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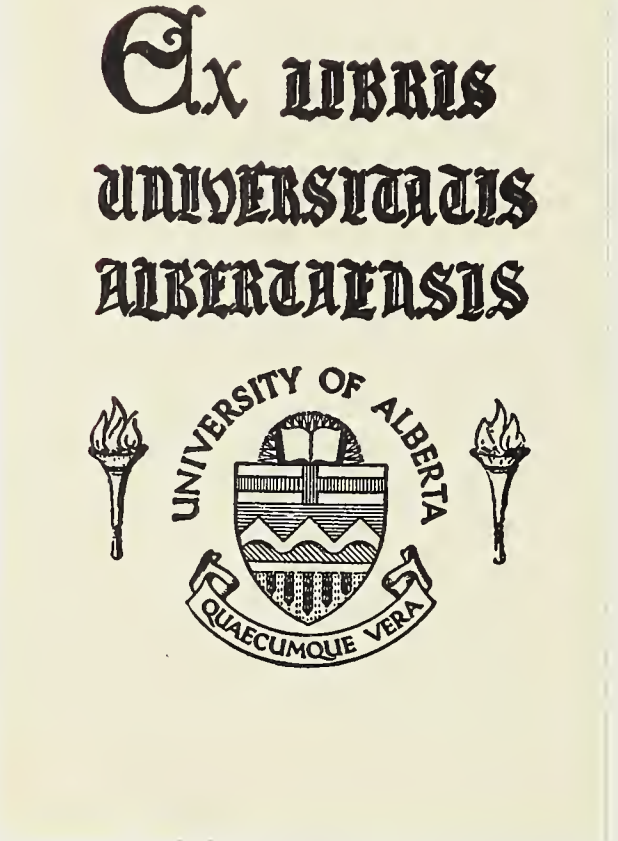

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THE UNIVERSITY OF ALBERTA

MICROBIAL DEGRADATION OF THE SCLEROTIA OF TWO PHYTOPATHOGENIC FUNGI IN THE SOIL

A DISSERTATION

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF PLANT SCIENCE

by

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ABSTRACT

A comparative study of the behavior of the sclerotia of two phytopathogenic fungi, namely Claviceps purpurea (Fr.) Tulasne and Sclerotinia Sclerotiorum (Lib.) D By was made in soil.

Milk agar proved the best medium and 20°C. the best temperature for sclerotia production by S. sclerotiorum. Agar-coated glass tubes increased the yield and size of the sclerotia over glass beads and glass slides added to agar cultures of this fungus in Petri plates.

When treated (with $HgCl_2$), treated and washed and untreated sclerotia of both fungi were planted in natural soil and in natural soil frozen and thawed before planting the sclerotia of both fungi were attacked by soil micro-organisms. The percentage of visible area of the sclerotia attacked was highest in the treated group and lowest in the untreated group.

As a result of the attack of soil micro-organisms the sclerotia of C. purpurea failed to germinate to the perfect stage and all the sclerotia of the treated and the treated and washed group were attacked in natural soil. Under the same conditions some sclerotia of the untreated group remained unattacked. The same behavior was observed in frozen and thawed soil except that some sclerotia of all groups and especially of the untreated group, remained unattacked, and some of the unattacked sclerotia germinated to the perfect stage.

The sclerotia of S. sclerotiorum also did not germinate as a result of the attack of soil micro-organisms in natural soil but most of them remained unattacked in all treatments. Some of those unattacked

germinated to the perfect stage. Similar results were obtained in frozen and thawed soil except that the number of sclerotia attacked was less than in natural soil. Untreated sclerotia were not attacked in either soil.

The sclerotia of C. purpurea proved much more suceptible to the attack of soil micro-organisms than those of S. sclerotiorum.

'Black bodies' resembling new sclerotia were formed on some of the original sclerotia of S. sclerotiorum in natural soil in all treatments. Peeled sclerotia of S. sclerotiorum formed 'secondary' and 'pseudo' sclerotia in natural soil. Peeled sclerotia of C. purpurea were more suceptible to the attack of soil micro-organisms than unpeeled ones. Treated sclerotia both normal and peeled of C. purpurea were degraded earlier than untreated ones.

Soil fungi belonging to the genera Gliocladium, Penicillium, Fusarium Trichoderma and Aspergillus were found attacking the sclerotia of both fungi in natural and frozen and thawed soil but Gliocladium was the predominant genus present.

Gliocladium sp. was shown to be parasitic on the sclerotia of both C. purpurea and S. sclerotiorum. It inhibited their germination to the perfect stage and caused their degradation. This was demonstrated by artificial inoculation of uncontaminated sclerotia produced in the case of C. purpurea by a hot water treatment at 140°F. devised for the purpose.

The sclerotia of S. sclerotiorum germinated to form mycelium and 'secondary' sclerotia in sterilized natural soil.

ACKNOWLEDGEMENTS

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INTRODUCTION

This study is concerned with the comparative behavior of the sclerotia of two quite different plant pathogenic fungi namely Claviceps purpurea (Fries) Tulasne and Sclerotinia sclerotiorum (Lib) DBy in the soil.

The former causes the floral disease of rye, wheat, barley and many other grasses known as Ergot while the latter attacks numerous dicotyledonous field and horticultural crops and many weeds causing rots of roots, stems and other parts. Both parasites are responsible for extensive losses.

Sclerotia are vegetative structures consisting of hardened masses of mycelium of various shapes and sizes usually with dark outer coatings. Those of C. purpurea are elongated and horn-like, while those of S. sclerotiorum are roughly spherical or irregular in shape. These structures withstand heat, cold and other adverse conditions and play important roles in the life cycles of fungi producing them.

The sclerotia of C. purpurea are formed in the inflorescences of its host plants. They fall to the ground or they are harvested with the seed and are often sown with the seed. The sclerotia of S. sclerotiorum are formed mainly on or in the basal parts of diseased host plants and are left in the soil when these parts decay.

The sclerotia of C. purpurea germinate to produce stromata bearing perithecia containing ascospores. This commonly takes place on or near the soil surface. Those of S. sclerotiorum in similar positions

form apothecia bearing ascospores and also may give rise to mycelium. The ascospores of both fungi may infect their hosts and cause disease. The mycelium of S. sclerotiorum also can infect its hosts and cause disease.

SCOPE AND PURPOSE OF PRESENT STUDIES

The present studies were made to compare the behavior of the sclerotia of two phytopathogenic fungi namely C. purpurea and S. sclerotiorum in the soil. These sclerotia are commonly deposited on or in the soil where they are subjected to the physical, chemical and biological factors of the soil environment. The influence on them of soil biological factors has been given chief attention in these studies.

The main purpose of this investigation has been to determine (1) the comparative degrading effect of the general microflora of natural soil on the sclerotia and (2) the specific effect of one soil fungus on them.

PART I

COLLECTION OF MATERIAL

Since considerable quantities of sclerotia of the two fungi under investigation were needed, supplies had to be obtained in advance of the initiation of the experimental work. Those of C. purpurea were collected from naturally infected or from artificially inoculated hosts while those of S. sclerotiorum were obtained from cultures on artificial media.

A. Collection of Sclerotia of C. purpurea

Some of the sclerotia of C. purpurea used were collected in the fall of 1958 from naturally infected Prolific spring rye (Secale cereale L.) plants grown in field plots at the University of Alberta in Edmonton. After drying at room temperature for a day they were stored in a refrigerator in cotton plugged flasks at approximately 5°C.

Additional sclerotia of C. purpurea were collected in 1959 from heads of Prolific spring rye which had been inoculated in the flowering stage with conidia of the pathogen. Inoculations were made between 8 and 10 a.m. (Fushtey) (4) on three consecutive days during the second week of July.

The inoculum used was obtained by growing the fungus on potato dextrose agar slants for ten days at room temperature. A suspension of conidia from three slants in 120 ml. of distilled sterilized water was used for each inoculation. Fresh inoculum was prepared each day and a hand atomizer was used in spraying the rye heads. Sclerotia, which

were produced in abundance, were collected in the fall and stored in the same manner as those obtained from naturally infected plants.

B. Collection of Sclerotia of S. sclerotiorum

Sclerotia of S. sclerotiorum were obtained from cultures grown on various artificial media. The isolate used was obtained from A. R. McKenzie, Graduate Research Assistant, of this laboratory, who isolated it from a diseased flax stem. The culture was maintained on slants of potato dextrose agar (P.D.A.) in test tubes at room temperature.

The following general routine and special procedures were adopted in order to produce the sclerotia.

(a) General routine procedure

Sterilized 2 x 9 cm. Petri dishes were used as culture chambers. Approximately 15 ml. of P.D.A. were poured into each dish. After the medium hardened it was sown with mycelium of the pathogen. After it had grown at room temperature for 30 days sclerotia were collected from the plates. These, after drying at room temperature for a day were stored in an incubator in cotton-plugged flasks or tubes. The general procedure was carried out from September 1958 to September 1959.

(b) Special procedures

In these procedures different artificial media were tested and different techniques were developed. The main aim was to get as many sclerotia as possible with the least effort. These special procedures are discussed in detail in the following sections.

i) Different artificial media

Four artificial media viz. potato dextrose agar, potato sucrose agar, corn meal agar and milk agar were compared for their effectiveness in encouraging sclerotia formation by S. sclerotiorum at nine different temperatures, i.e. 0, 5, 10, 15, 20, 25, 30, 35 and 40°C. The procedure was as follows:-

(1) To each of a number of half pint sized dairy milk bottles 25 ml. of medium were added. The the bottles were sterilized for 15 minutes at 15 lbs. pressure in an autoclave.

(2) When the medium in each bottle was hard, mycelium from a 7-day culture of the fungus, which had been incubated at 20°C. in a Petri dish, was sown aseptically in the centre of it. The inoculum consisted of a uniform disc of agar bearing mycelium cut by a No. 1 cork borer aseptically from a Petri dish culture.

(3) The bottles were placed in dark incubators held at different temperatures, where they were kept for 30 days. At each temperature, 4-5 replications of the fungus on each medium were incubated.

(4) Following the incubation period the sclerotia were collected from the bottles and dried at room temperature for a day. Then the weight and number of sclerotia were recorded. The results summarized in Table No. 1 show that:-

(1) Milk agar proved to be the best medium for sclerotia production and corn meal agar the poorest.

(2) Potato dextrose agar and potato sucrose agar media proved more or less alike for sclerotia production.

Table 1 - Effect of different artificial media on the production of sclerotia of *Sclerotinia sclerotiorum* at different temperatures[1]

Temperature in °C.	Potato Dextrose Agar		Potato Sucrose Agar		Corn Meal Agar		Milk Agar	
	Number	Wt. in Gm.	Number	Wt. in Gm.	Number	Wt. in Gm.	Number	Wt. in Gm.
5	NS		NS		NS		NS	
10	6.6	0.110	5.0	0.174	6.8	0.040	8.3	0.990
15	6.4	0.239	7.2	0.185	6.4	0.036	11.3	1.007
20	13.0	0.112	9.8	0.127	4.0	0.034	37.5	0.947
25	6.5+	0.034+	17.8++	0.132++	NS		16.5	0.826
30	NS		NS		NS		NS	
TOTAL	32.5	0.495	39.8	0.618	17.2	0.110	73.6	3.770

NS - No Sclerotia
\+ - Average of two replications
++ - Average of four replications

[1]The figures are averages of 5 replications except for milk agar where they are averages of 4 replications.

(3) Sclerotia were not formed on potato dextrose agar, potato sucrose agar, corn meal agar and milk agar at 0°, 5°, 30°, 35°, and 40°C.

(4) Sclerotia were not formed on corn meal agar at 25°C.

(5) The best temperature for producing numbers of sclerotia varied with the medium. For potato-dextrose agar it was 20°C., for potato sucrose agar 25°C., for corn meal agar 10°C., and for milk agar 20°C. The best temperature for producing heavy sclerotia also varied with the medium. For potato dextrose agar it was 15°C., for potato sucrose agar 15°C., for corn meal agar 10°C. and milk agar 15°C.

ii) Different techniques for increasing the yield of sclerotia

Glass beads, glass slides and glass tubes were employed in different techniques. The principle of these techniques was to increase the growing surface of the Petri dish. It was thought that the greater mass of mycelium thus obtained might result in increased yields of sclerotia of S. sclerotiorum.

(1) Glass beads

Since in a preliminary experiment with steel beads no visible growth of the fungus occurred and no sclerotia were formed, it was decided to experiment with solid glass beads (1.5 cm. in diameter). It was found, using procedures outlined below, that for both mycelium and sclerotia production glass beads were much superior to steel beads. It is of interest that Santoro and Casida (1959) (15) found glass beads useful in improving the vegetative growth of mycorrhizal and slow-growing fungi. In the present studies glass beads were tested (Fig. 1)

in the following manner:

1. Deep Petri dishes (3 x 9 cm.) were employed as culture chambers and 15 beads were placed in each.

2. The dishes containing the beads were sterilized at 15 lbs. pressure for 15 minutes in an autoclave.

3. The sterilized dishes were stored at 0° in a walk-in cold room for a day.

4. The desired amount of P.D.A. (different amounts from 13 ml. to 20 ml. per dish were used) was poured into the cold Petri dishes aseptically so that the whole beads were well immersed in it. The medium stuck well to the cold beads.

5. After hardening of the medium it was sown in the centre with 7-day old mycelium of the fungus which had been grown on P.D.A. at 20°C. in Petri dishes.

6. An equal amount of inoculum cut from the Petri dish cultures with a No. 1 cork borer was added to all dishes.

7. The controls consisted of similarly prepared dishes without beads.

8. The inoculated dishes were stored at room temperature and the fungus was allowed to grow for 30 days before data were taken.

9. The sclerotia were dried for a day at room temperature before recording data. The results are recorded in Table No. 2.

Table shows that:

1. The weight of sclerotia produced in dishes containing

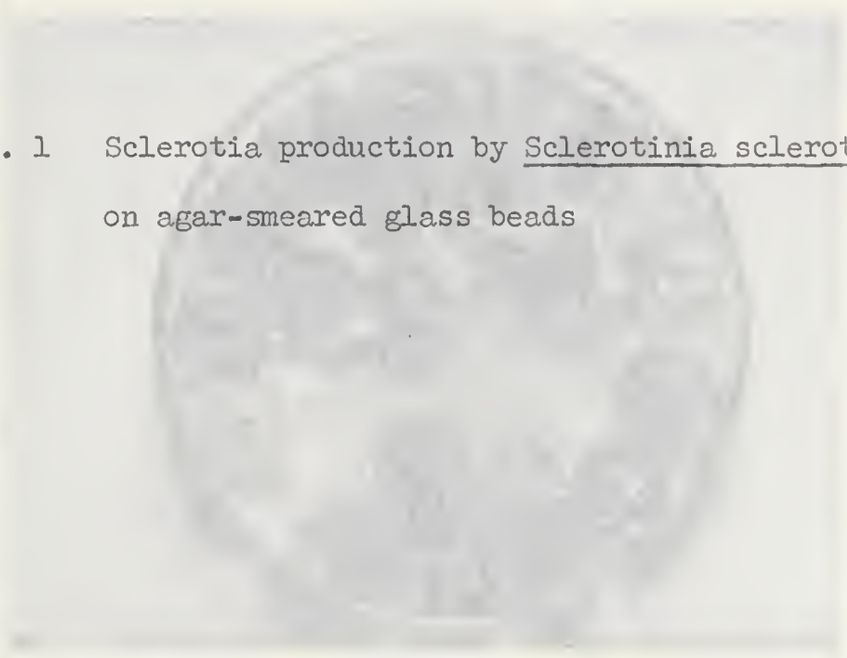


Fig. 1 Sclerotia production by Sclerotinia sclerotiorum on agar-smeared glass beads

Fig. 1 Solenoid production by Heterotinia cerebriformis on agar-smeared glass beads

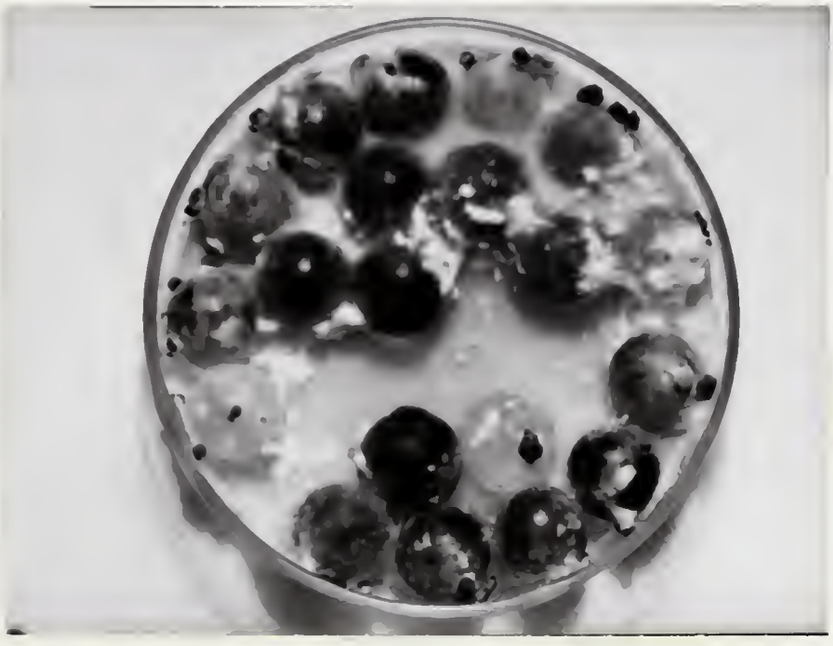


Table 2 - Effect of the addition of glass beads to culture dishes on the yield of sclerotia of Sclerotinia sclerotiorum

No.	P.D.A. used per plate in ml.	Without Beads		Average No. of Replications	With Beads		Average No. of Replications
		Number	Wt. in Gm.		Number	Wt. in Gm.	
1	13	20.1	0.060	9	12.6	0.071	9
2	15	25.3	0.097	6	14.8	0.114	6
3	17	25.3	0.104	6	17.3	0.114	6
4	20	28.0*	0.119*	6	16.2*	0.148*	6

* One replication contaminated.

Table 3 - Comparative effectiveness of spherical (without bottom) and flattened (with bottom) glass beads added to culture dishes on the yield of sclerotia of Sclerotinia sclerotiorum

No.	P.D.A. used per plate in cc.	Without Bottom		Average No. of Replications	With Bottom		Average No. of Replications
		Number	Wt. in Gm.		Number	Wt. in Gm.	
1	13	11.0	0.059	4	16.0	0.057	4
2	15	14.2	0.091	5	13.6	0.119	5
3	17	20.0	0.172	4	20.5	0.163	4
4	20	28.5	0.192	4	22.7*	0.161*	4

* One replication contaminated.

glass beads was consistently higher than that of sclerotia in control plates whereas the reverse was true for the number of sclerotia.

2. In the range of amounts of medium per plate used, 20 ml. was in general the optimum amount for sclerotia production. This is further supported by the result in Table 3.

The sclerotia formed in bead plates were larger than those in control plates.

One drawback in using beads is that they tend to run together thus reducing the growing area of the fungus. In order to remove this drawback, individual beads were flattened on one side with an electric grinder so that they would sit separately without running together, i.e. a bottom was made on each bead. The bottomed beads were tested for sclerotia production, using 18 beads in each Petri dish (3 x 9 cm.). Control dishes contained beads without bottoms. The procedure of the previous experiment was followed. From Table 3 which gives the results obtained, it will be noted that the use of flattened over spherical beads did not increase either the number or the weight of sclerotia produced.

(2) Glass slides

Microscopic glass slides (0.1 x 2.5 x 5.7 cm.) were employed in the following trial in an attempt to increase sclerotia production by S. sclerotiorum. The procedures employed were as follows:-

1. Sterilized storage Petri dishes 6 x 9 cm. were poured aseptically with 50 ml. P.D.A. Twelve oven-sterilized slides were dipped in 20 ml. melted P.D.A. and inserted in the hardened medium in each

Petri dish. (Fig. 2). The medium remaining out of the 20 ml. P.D.A. was poured in the same Petri dish.

2. After the medium had hardened, it was sown in the centre with 7-day old mycelium of the fungus using the same amount of inoculum for all dishes.

3. After the mycelium had grown at room temperature for 30 days, sclerotia were collected. They were dried at room temperature for a day and the data recorded. These are given in Table No. 4.

4. Control plates had no slides.

Table No. 4 shows that a larger number of sclerotia was produced in the Petri dishes with slides but their average weight was less compared with that of the sclerotia formed in the absence of slides.

(3) Glass tubes

Glass tubes with dimensions of 6.4 x 0.7 (I.D.), 0.9 (O.D.) cm. were used in attempting to increase sclerotia production in much the same way as glass slides were employed in the previous trial. The control plates had no tubes. The results are shown in Table No. 5.

Table No. 5 shows that more and bigger sclerotia were produced in Petri dishes with tubes than in those without tubes.

Table 4 - Sclerotia production by Sclerotinia sclerotiorum in culture dishes with and without glass slides.

No.	Replication Number	Without Slides		With Slides	
		Number	Wt. in Gm.	Number	Wt. in Gm.
1	1	56.0	0.795	89.0	0.591
2	2	77.0	0.883	96.0	0.789
3	3	62.0	0.802	80.0	0.591
4	4	74.0	0.838	Contaminated	
Average		67.3	0.817	88.3	0.726

Table 5 - Sclerotia production by Sclerotinia sclerotiorum in culture dishes with and without glass tubes.

No.	Replication Number	Without Tubes		With Tubes	
		Number	Wt. in Gm.	Number	Wt. in Gm.
1	1	47.0	0.493	70.0	0.463
2	2	51.0	0.432	73.0	0.511
3	3	42.0	0.391	58.0	0.433
4	4	Contaminated		Contaminated	
Average		46.7	0.439	67.0	0.469

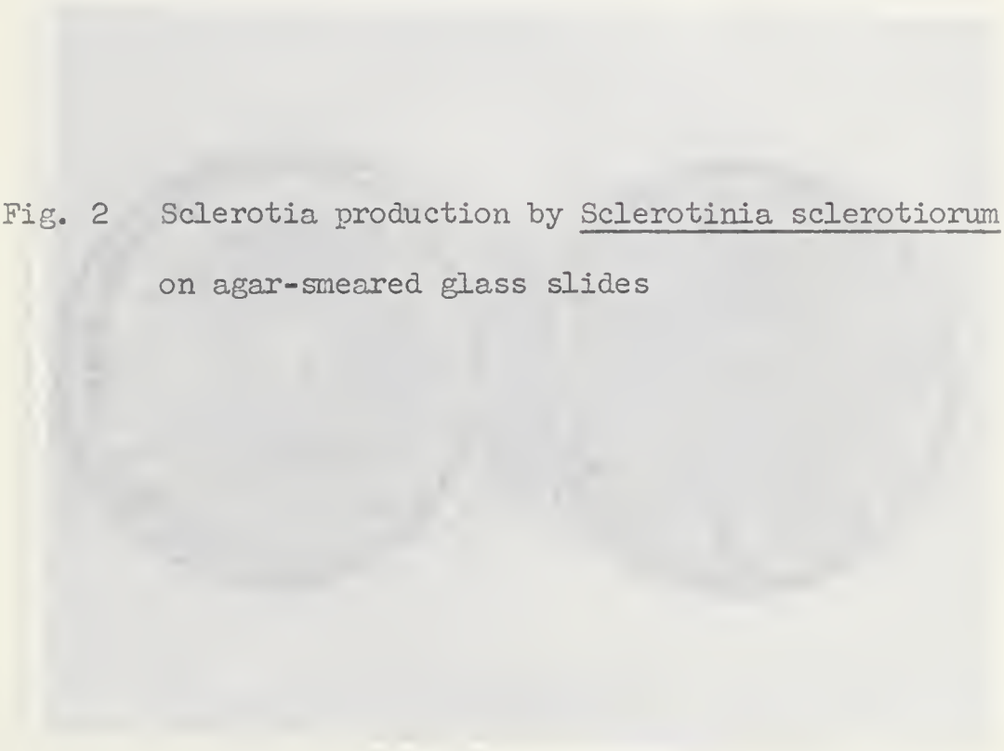
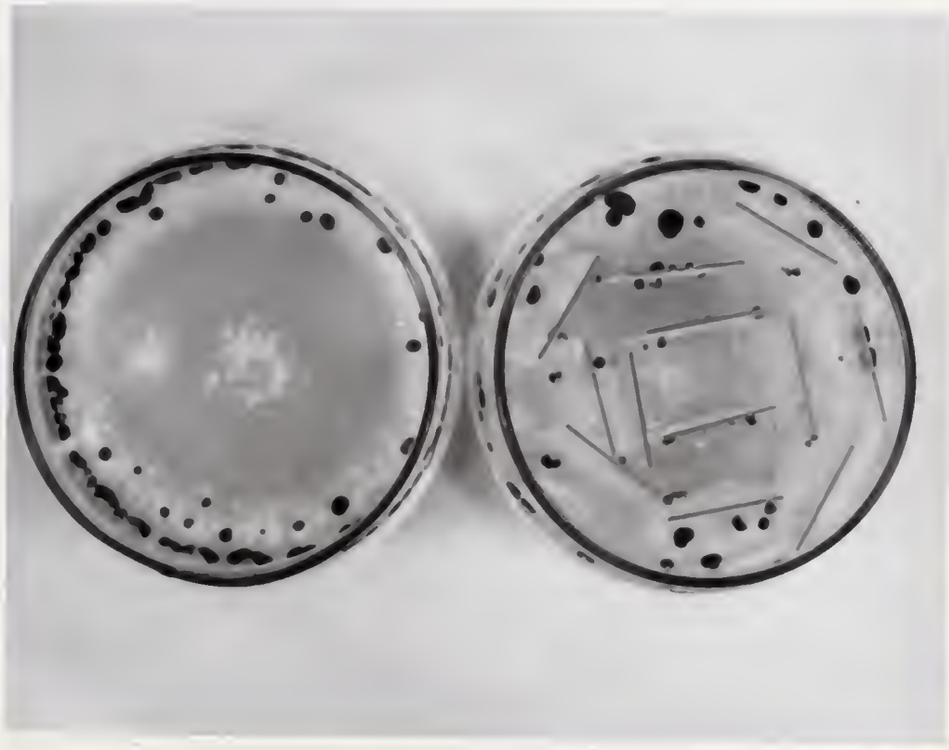


Fig. 2 Sclerotia production by Sclerotinia sclerotiorum on agar-smeared glass slides



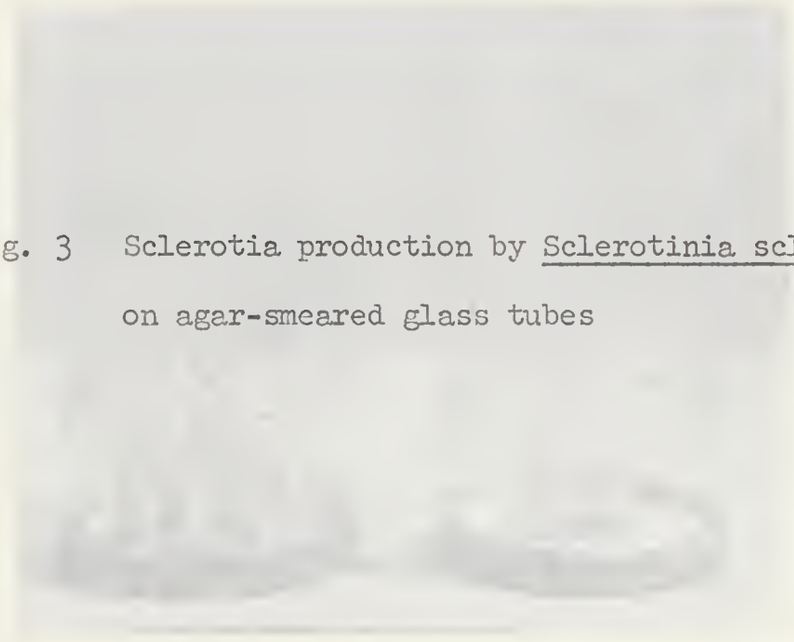


Fig. 3 Sclerotia production by Sclerotinia sclerotiorum on agar-smeared glass tubes

Fig. 3 Colorado production by [illegible]
on sput-covered glass tubes

P A R T II

EXPERIMENTS ON BEHAVIOR OF SCLEROTIA IN THE SOIL

Experiments were undertaken to study the behavior of the sclerotia of Claviceps purpurea (Fr.) Tulasne and Sclerotinia sclerotiorum (Lib.) D.By in the soil, giving particular attention to their germination and degradation by soil micro-organisms.

Previous Work Done

Henry (1955) (8) has shown that the sclerotia of Claviceps purpurea (Fr.) Tulasne germinate poorly or not at all, as a result of attack of soil micro-organisms, if they are planted in natural black soil in Petri dishes at 15°C. after treating them with a 1:1000 aqueous solution of mercuric chloride for five minutes.

Materials And Methods

Sclerotia of C. purpurea and S. sclerotiorum collected as described in Part I were used in all the experiments in this part.

The same procedure was followed in the different experiments except for a few variations which are mentioned in the discussion of individual experiments. The general procedure was as follows:-

Black, air dry soil was added to deep Petri dishes (3 x 9 cm.) at the rate of 55 gm. per dish. The soil was levelled and moistened evenly with distilled water by means of a burette. The amount of moisture added was the same for all dishes used in one experiment. It

varied from one set of experiments to another and was dependent on the moisture condition of the soil.

The sclerotia were planted on the soil surface and pushed slightly into it but not covered. Those of Claviceps purpurea were placed in horizontal position on the soil. Before planting the sclerotia were either treated, treated and washed or left untreated. Treatment consisted of immersing the sclerotia for one minute in 30 ml. of a 1:1000 aqueous solution of mercuric chloride. Treated and washed sclerotia were treated in the same way but in addition were washed immediately in three separate lots of sterilized distilled water. Twenty-five ml. of sterilized distilled water were used per wash. The prepared Petri dishes were incubated at 15°C.

Series 1 - Behavior of Sclerotia of C. purpurea in Natural Soil

Experiment 1

In this experiment natural black soil from north of Edmonton was used and moistened with 27 ml. of distilled water per Petri dish. Three dishes each containing five sclerotia of treated, three of treated and washed and three of untreated sclerotia were planted. The sclerotia were examined at intervals for germination (production of stromata) and for the extent of their contamination by micro-organisms. The first observation was made after about 6 weeks (39 days) and second after about 8 weeks (54 days). The results are shown in Table No. 6. Since none of the sclerotia germinated, only the extent of their contamination is recorded in the table.

Table 6 - Percentage of visible area of sclerotia of C. purpurea attacked by micro-organisms in natural soil

TREATED

First Observation

No.	Replication Number	1	2	3	4	5
1	1	100	100	100	100	100
2	2	100	90	95	90	80
3	3	100	100	95	80	80

Second Observation

No.	Replication Number	1	2	3	4	5
1	1	100	100	100	100	100
2	2	95	100	100	100	100
3	3	100	100	100	100	95

TREATED AND WASHED

First Observation

No.	Replication Number	1	2	3	4	5
1	1	100	100	100	85	80
2	2	100	100	100	100	100
3	3	100	85	75	90	90

Second Observation

No.	Replication Number	1	2	3	4	5
1	1	100	100	100	100	85
2	2	100	100	95	95	90
3	3	100	100	100	100	90

UNTREATED

First Observation

No.	Replication Number	1	2	3	4	5
1	1	0	20	40	50	40
2	2	15	15	30	35	45
3	3	0	10	45	80	35

Second Observation

No.	Replication Number	1	2	3	4	5
1	1	90	85	70	0	30
2	2	80	85	70	65	85
3	3	0	30	40	40	65

Average of percentages of visible area of sclerotia of C. purpurea attacked by micro-organisms in each treatment.

No.	Treatment	First Observation	Second Observation
1	Treated	94.0	99.3
2	Treated and Washed	93.7	97.0
3	Untreated	30.7	55.7

Table No. 6 shows that:-

(1) All the sclerotia were attacked by micro-organisms except 2 in the untreated series.

(2) The percentage of visible area attacked by micro-organisms was highest in the treated and lowest in the untreated sclerotia (Fig. 4).

Experiment 2

In another experiment the sclerotia behaved in general in the same way. In this experiment black soil from the West Field of the University of Alberta was used and 25 ml. of distilled water was used to moisten it per Petri dish. Six replications of each treatment were planted in this experiment. Each sclerotium was marked by a sterilized straight iron pin, placed in the soil beside it. The pins were sterilized by dipping in 95% ethyl alcohol and flaming in a Bunsen flame. Care was taken to plant sclerotia which were entire, that is, those with both tail and head.

It was observed soon after planting that the tail of the sclerotium is attacked first by micro-organisms followed by the head. (Fig. 5). The tail as designated here is the part which was attached to the plant and is yellowish-white.

The sclerotia were examined for germination (production of stromata) and for the extent of their contamination by micro-organisms. The first observation was made after 6 weeks (42 days) and the second after 8 weeks (56 days). The results are shown in Table No. 7. The sclerotia which were examined for germination by the production of stromata but none was germinated. The extent of their contamination is recorded in the table.

Fig. 4 Behavior of sclerotia of Claviceps purpurea in natural soil. l. to r. treated, treated and washed, and untreated

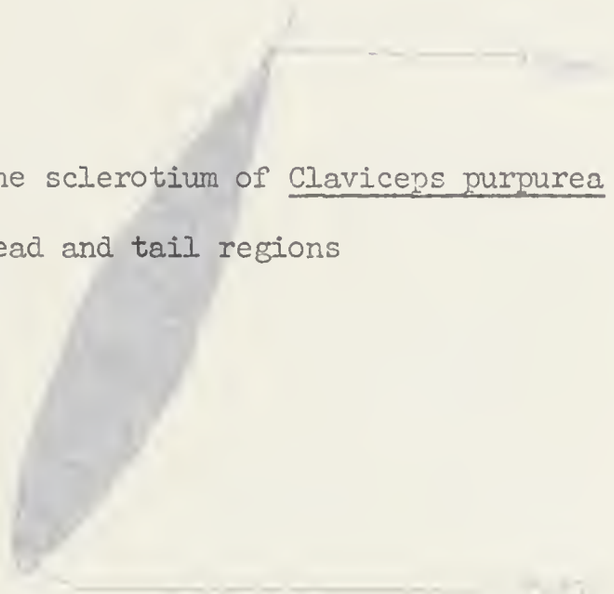


Fig. 5 The sclerotium of Claviceps purpurea showing head and tail regions

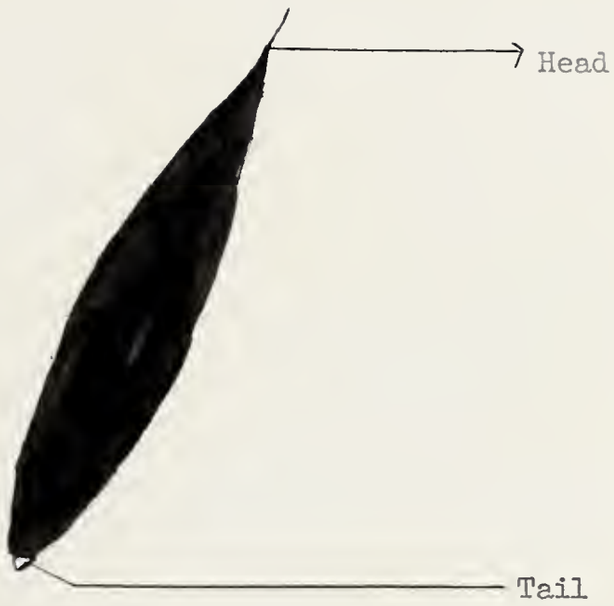

Head
Tail

Table 7 - Percentage of visible area of sclerotia of C. purpurea attacked by micro-organisms in natural soil

TREATED

First Observation

No.	Replication Number	1	2	3	4	5
1	1	100	90	70	40	30
2	2	90	100	100	90	50
3	3	100	90	90	80	60
4	4	90	90	80	100	80
5	5	100	100	100	95	30
6	6	90	90	100	90	30

Second Observation

No.	Replication Number	1	2	3	4	5
1	1	100	100	100	100	100
2	2	100	100	100	100	100
3	3	95	85	90	70	40
4	4	100	100	100	100	100
5	5	100	100	100	100	100
6	6	100	100	100	100	40

TREATED AND WASHED

First Observation

No.	Replication Number	1	2	3	4	5
1	1	100	90	80	60	40
2	2	100	100	80	5	50
3	3	60	50	20	20	10
4	4	50	50	30	80	80
5	5	100	80	100	100	80
6	6	80	80	80	70	80

Second Observation

No.	Replication Number	1	2	3	4	5
1	1	100	100	100	100	100
2	2	90	70	80	95	70
3	3	100	95	90	90	40
4	4	80	35	30	50	70
5	5	80	30	30	40	70
6	6	80	40	40	40	30

UNTREATED

First Observation

No.	Replication Number	1	2	3	4	5
1	1	80	0	0	0	0
2	2	0	0	0	30	70
3	3	20	0	0	0	0
4	4	0	0	0	0	100
5	5	0	0	0	0	0
6	6	40	0	0	0	0

Second Observation

No.	Replication Number	1	2	3	4	5
1	1	0	0	0	0	0
2	2	0	0	0	0	100
3	3	0	0	0	60	75
4	4	40	0	0	0	0
5	5	0	0	0	0	40
6	6	0	0	0	0	100

Average of percentages of visible area of sclerotia of *C. purpurea* attacked by micro-organisms in each treatment

No.	Treatment	First Observation	Second Observation
1	Treated	81.5	94.0
2	Treated and Washed	66.8	68.8
3	Untreated	11.3	13.8

The table shows that:-

(1) All the sclerotia were attacked by micro-organisms except 24 in untreated group.

(2) The percentage of visible area attacked by micro-organisms was highest in the treated and lowest in the untreated sclerotia.

Some mites were seen on some of the sclerotia. They were identified as Rhizoglyphus sp.

Series 2 - Behavior of Sclerotia of S. sclerotiorum in Natural Soil

Experiment 1

In this study black soil from north of Edmonton was placed in deep Petri dishes moistened and planted with treated (with mercuric chloride), treated and washed and untreated sclerotia of S. sclerotiorum as in the previous experiment with C. purpurea. Seventeen ml. of distilled water per plate was used to moisten the soil. Each plate was planted with nine sclerotia and each treatment was replicated three times.

The first observation was made after about 9 weeks (65 days) and the second after about 11 weeks (80 days). Similar records were made as for C. purpurea in previous experiments. The results are shown in Table No. 8.

Table 8 - Percentage of visible area of sclerotia of S. sclerotiorum attacked by micro-organisms in natural soil

TREATED

First Observation

No.	Replication Number	1	2	3	4	5	6	7	8	9
1	1	100	40	0	0	0	0	0	0	0
2	2	80	0	0	0	0	0	0	0	0
3	3	90	0	0	0	0	0	0	0	0

Second Observation

No.	Replication Number	1	2	3	4	5	6	7	8	9
1	1	100	40	10	0	0	0	0	0	0
2	2	80	0	0	0	0	0	0	0	0
3	3	90	0	0	0	0	0	0	0	0

TREATED AND WASHED

First Observation

No.	Replication Number	1	2	3	4	5	6	7	8	9
1	1	90	0	0	0	0	0	0	0	0
2	2	0	0	0	0	0	0	0	0	0
3	3	75	0	0	0	0	0	0	0	0

Second Observation

No.	Replication Number	1	2	3	4	5	6	7	8	9
1	1	0	0	0	0	0	0	0	0	0
2	2	95	0	0	0	0	0	0	0	0
3	3	75	0	0	0	0	0	0	0	0

UNTREATED

First Observation

No.	Replication Number	1	2	3	4	5	6	7	8	9
1	1	0	0	0	0	0	0	0	0	0
2	2	0	0	0	0	0	0	0	0	0
3	3	0	0	0	0	0	0	0	0	0

Second Observation

No.	Replication Number	1	2	3	4	5	6	7	8	9
1	1	0	0	0	0	0	0	0	0	0
2	2	0	0	0	0	0	0	0	0	0
3	3	0	0	0	0	0	0	0	0	0

Average of percentages of visible area of sclerotia of S. sclerotiorum attacked by micro-organisms in each treatment.

No.	Treatment	First Observation	Second Observation
1	Treated	11.5	11.9
2	Treated and Washed	6.1	6.3
3	Untreated	0.0	0.0

The Table No. 8 shows that:-

(1) Only 5 sclerotia of treated and 2 of treated and washed were attacked by micro-organisms, also that none of the untreated sclerotia were attacked.

(2) The percentage of visible area attacked by micro-organisms was highest for treated and lowest for untreated sclerotia.

When the second observation was made some sclerotia in all treatments were found germinating by the production of fruiting-body stipes (Fig. 6). However, since the plates had been kept in the dark no apothecial cups had formed but later, when put in a light, near a window at room temperature, apothecial cups were formed. (Fig. 7). They were watered with distilled water frequently with their covers on. The results are in contrast to those for C. purpurea in which case all the treated sclerotia were attacked by micro-organisms.

Experiment 2

In another similar experiment black soil from the West Field of the University of Alberta was used. Five sclerotia were planted in each plate instead of 9. It was observed after 42 days that the outer surfaces of some of the sclerotia did not appear to be attacked by micro-organisms. Such sclerotia in general had no observable outer covering of micro-organisms. It was noted however, by probing with a needle, that such sclerotia were partially or wholly softened or hollow as the needle penetrated them very easily. These sclerotia were called 'degraded'. Such sclerotia were observed in all treatments. Table No. 9 shows the number of 'degraded' sclerotia in each treatment.

Fig. 6 Photograph showing l. degradation of sclerotia of Claviceps purpurea in natural soil, r. germinating sclerotia of Sclerotinia sclerotiorum

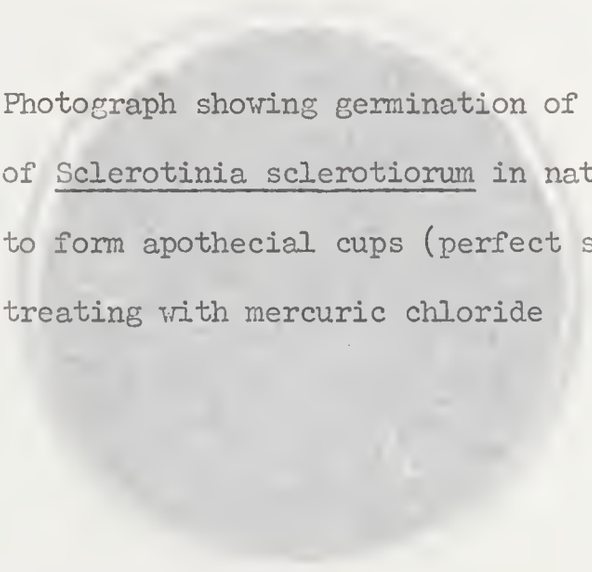


Fig. 7 Photograph showing germination of sclerotia of Sclerotinia sclerotiorum in natural soil to form apothecial cups (perfect stage) after treating with mercuric chloride

Fig. 1 Photograph showing germination of sclerotia of Sclerotinia sclerotiorum in natural soil to form apothecial cups (perfect stage) after treating with mercuric chloride

Table No. 9 - Number of 'degraded' sclerotia of S. sclerotiorum in each treatment in natural soil

No.	Number of Sclerotia Planted	Treatment	Number of 'Degraded' Sclerotia First Observation (After 42 days)	Second Observation (After 65 days)
1	30	Treated	2	17
2	30	Treated and Washed	1	14
3	30	Untreated	1	17

After 65 days it was observed that each of a number of the degraded sclerotia formed a 'black body' on or adjacent to the original sclerotium. This occurred in each treatment. Table No. 10 shows the number of 'black bodies'. (Fig. 8).

Table No. 10 - Number of 'black bodies' formed on 'degraded' sclerotia of S. sclerotiorum in each treatment in natural soil

No.	Number of Sclerotia Planted	Treatment	Number of 'Black Bodies' First Observation (After 42 days)	Second Observation (After 65 days)
1	30	Treated	0	2
2	30	Treated and Washed	0	1
3	30	Untreated	0	4

'Black bodies' formed on the degraded sclerotia of S. sclerotiorum have to the writer's knowledge not been observed before. They may be 'secondary' or 'pseudo' sclerotia. Further investigation on this point is needed.

Series 3 - Behavior of Sclerotia of C. purpurea and S. sclerotiorum together in Natural Soil

Experiment 1

In this series of studies treated (with $Hgcl_2$), treated and washed and untreated sclerotia of both of the above fungi were planted in natural soil in the same Petri plates at the same time. Otherwise the procedure followed was similar to that of the preceding experiment.

Notes on the percentage of area of the sclerotia visibly attacked by micro-organisms and on sclerotia germination were taken about 5 and about 7 weeks after planting. The results are shown in Table No. 11.

Table No. 11 - Percentage of visible area of sclerotia of C. purpurea and S. sclerotiorum attacked by micro-organisms when grown together in natural soil in the same plate

TREATED

SCLEROTIA OF C. PURPUREA - First Observation

No.	Replication Number	1	2	3	4	5
1	1	95	90	85	80	80
2	2	90	85	40	45	60
3	3	95	100	40	40	60

SCLEROTIA OF S. SCLEROTIORUM - First Observation

No.	Replication Number	1	2	3	4	5
1	1	100	95	0	80	70
2	2	100	0	0	0	100
3	3	100	100	0	20	20

SCLEROTIA OF C. PURPUREA - Second Observation

No.	Replication Number	1	2	3	4	5
1	1	100	100	95	90	95
2	2	95	90	90	95	30
3	3	100	100	100	70	60

SCLEROTIA OF S. SCLEROTIORUM - Second Observation

No.	Replication Number	1	2	3	4	5
1	1	100	100	100	0	0
2	2	100	100	0	0	0
3	3	100	100	100	100	0

TREATED AND WASHED

SCLEROTIA OF C. PURPUREA - First Observation

No.	Replication Number	1	2	3	4	5
1	1	85	95	75	50	50
2	2	100	10	40	95	85
3	3	95	70	80	50	80

SCLEROTIA OF S. SCLEROTIORUM - First Observation

No.	Replication Number	1	2	3	4	5
1	1	0	0	0	0	100
2	2	0	0	0	0	100
3	3	0	0	60	100	100

SCLEROTIA OF C. PURPUREA - Second Observation

No.	Replication Number	1	2	3	4	5
1	1	95	90	90	70	40
2	2	100	95	85	40	10
3	3	95	95	100	85	85

SCLEROTIA OF S. SCLEROTIORUM - Second Observation

No.	Replication Number	1	2	3	4	5
1	1	0	0	0	0	100
2	2	0	0	100	100	60
3	3	0	0	0	0	100

UNTREATED

SCLEROTIA OF C. PURPUREA - First Observation

No.	Replication Number	1	2	3	4	5
1	1	40	5	0	0	0
2	2	5	10	10	15	30
3	3	0	0	5	5	20

SCLEROTIA OF S. SCLEROTIORUM - First Observation

No.	Replication Number	1	2	3	4	5
1	1	0	0	0	100	100
2	2	0	0	0	0	0
3	3	0	0	0	0	0

SCLEROTIA OF C. PURPUREA - Second Observation

No.	Replication Number	1	2	3	4	5
1	1	0	0	10	10	50
2	2	40	20	20	30	90
3	3	0	0	0	90	40

SCLEROTIA OF S. SCLEROTIORUM - Second Observation

No.	Replication Number	1	2	3	4	5
1	1	0	0	0	0	0
2	2	0	0	0	0	0
3	3	100	100	100	0	0

(Table continued on next page)

The table shows that:-

(1) The percentage of visible area of the sclerotia attacked by micro-organisms was highest in treated sclerotia of both fungi.

(2) The percentage of visible area and the number of sclerotia attacked by micro-organisms was greater for Claviceps purpurea than for Sclerotinia sclerotiorum (Fig. 9).

Table No. 19 Continued

Averages of percentages of visible area of sclerotia of C. purpurea and S. sclerotiorum attacked by micro-organisms in each treatment

No.	Treatment	C. PURPUREA		S. SCLEROTIORUM	
		First Obs.	Second Obs.	First Obs.	Second Obs.
1	Treated	72.3	87.3	52.3	60.0
2	Treated and Washed	70.7	78.3	30.7	30.7
3	Untreated	9.7	26.7	13.3	20.0

Fig. 8 Formation of 'black bodies' (shown between pairs of pins) on some 'degraded' sclerotia of Sclerotinia sclerotiorum in natural soil

Fig. 8 Formation of 'black bodies' (drum between pairs of pins) on some 'degraded' auxotrophs of Sclerotinia sclerotiorum in natural soil

Fig. 9 Behavior of sclerotia of Claviceps purpurea and Sclerotinia sclerotiorum together in the same plate in natural soil. l. - r. treated and washed, treated and untreated

Fig. 9 Behavior of colonies of Clostridia Pasteurianum and Azotobacter chroococcum together in the same plate in natural soil. J. - r. treated and washed, heated and untreated

(3) All the sclerotia of C. purpurea were attacked by micro-organisms except 5 out of 15 in the untreated group.

(4) Only 9 out of 15 sclerotia of S. sclerotiorum of treated, 5 out of 15 of treated and washed, and 3 out of 15 of the untreated group were attacked by micro-organisms.

Series 4 - Behavior of Sections of Sclerotia of C. purpurea in Natural Soil

In previous tests, as has already been noted, it was observed that the tail of the sclerotium of C. purpurea, that is the yellowish part which was attached to the host, is more readily attacked by soil micro-organisms than any other part. In view of this observation, sclerotia were cut transversely into pieces and the behavior of the sections in natural soil was studied. In one test the sclerotia were cut in two and the two end pieces compared while in a second test only a central section was studied after removal of the two ends. Similar methods were followed as in the last two experiments.

After two weeks following planting it was observed that the cut surfaces of the pieces were readily attacked by micro-organisms in all the treatments. The sclerotia of C. purpurea, which are elongated and horny, are often broken into pieces in the threshing process and may be sown with the seed of rye and other grains. The fact that these broken ends are readily attacked by micro-organisms, suggests that they may be more readily degraded in the soil than normal unbroken sclerotia.

Series 5 - Behavior of Sections of Sclerotia of S. sclerotiorum in Natural Soil

Similar methods were followed as in the previous test in studying the behavior of sections of sclerotia of S. sclerotiorum in the soil. Completely black sclerotia from regular supplies were cut into two pieces and these were planted in natural soil. After two weeks following planting it was observed that the cut surfaces of the pieces were regularly attacked by micro-organisms in all the treatments.

As the sclerotia of this pathogen are generally formed on the lower parts of their host plants, and since they are commonly more or less spherical, the chances of them being broken into pieces are less than is the case with sclerotia of C. purpurea. Hence their chances of quick degradation in the soil are probably not as high as for the sclerotia of C. purpurea. Sometimes however they are produced in the inflorescences of certain host plants such as the sunflower, in which case an occasional one may be broken when the seed is threshed.

Series 6 - Behavior of Peeled Sclerotia of C. purpurea in Natural Soil

Experiment 1

It has already been reported that micro-organisms attack the cut surfaces of sclerotia of this fungus more readily than uncut surfaces. In view of these findings, the black outer layer of sclerotia was peeled off by means of a razor blade. This black layer is not uniform on the sclerotium nor is it easily removed, so only partial removal was possible.

It has been shown by MacFarland as cited by Gibson (7) that peeling the sclerotia of C. purpurea will not necessarily prevent them from germinating to produce the perfect stage. However it might encourage their degradation in natural soil. This possibility was therefore studied. The behavior of treated (with $Hgcl_2$), treated and washed and untreated in natural black soil was followed as was that of whole sclerotia and sections of sclerotia in preceding experiments.

Two observations were recorded. The first was made 3 weeks (21 days) and the second 5 weeks (35 days) after planting. After two weeks it was noted that micro-organisms were attacking the sclerotia. (Fig. 10). The results are shown in Table No. 12.

Table No. 12 - Percentage of visible area of peeled sclerotia of C. purpurea attacked by micro-organisms in natural soil

TREATED

First Observation

No.	Replication Number	1	2	3	4	5
1	1	95	100	95	85	70
2	2	100	95	95	90	75
3	3	100	95	90	30	60

Second Observation

No.	Replication Number	1	2	3	4	5
1	1	100	100	95	90	80
2	2	100	100	100	100	90
3	3	100	100	100	95	100

TREATED AND WASHED

First Observation

No.	Replication Number	1	2	3	4	5
1	1	80	5	80	90	60
2	2	100	100	20	50	20
3	3	90	95	80	70	10

Second Observation

No.	Replication Number	1	2	3	4	5
1	1	100	95	95	90	60
2	2	100	100	95	90	40
3	3	100	100	95	85	30

UNTREATED

First Observation

No.	Replication Number	1	2	3	4	5
1	1	60	0	0	0	0
2	2	1	20	20	60	10
3	3	0	1	10	15	5

Second Observation

No.	Replication Number	1	2	3	4	5
1	1	10	40	50	25	65
2	2	0	10	10	20	60
3	3	0	20	20	95	70

Average of percentages of visible area of peeled sclerotia of C. purpurea attacked by micro-organisms in each treatment

No.	Treatment	First Observation	Second Observation
1	Treated	85.0	96.7
2	Treated and Washed	63.3	85.0
3	Untreated	13.5	33.0

The table shows:-

(1) There was no germination of sclerotia whether they were treated or left untreated.

(2) All the sclerotia were attacked by micro-organisms except 2 in the untreated series.

(3) The percentage of visible area attacked by micro-organisms was highest in treated and lowest in untreated sclerotia.

Series 7 - Behavior of Peeled and Unpeeled Sclerotia of C. purpurea in Natural Soil

Experiment 1

In this series a study of the comparative behavior of peeled

Fig. 10 Behavior of peeled sclerotia of Claviceps purpurea in natural soil

Upper row - Treated and washed

Middle row - Treated

Lower row - Untreated

Fig. 10 Behavior of peeled sclerotia of Clavicops purpurea

in natural soil

Upper row - Treated and washed

Middle row - Treated

Lower row - Untreated

and unpeeled sclerotia of C. purpurea from the same sample was made in natural soil using the same procedure as in the previous experiment. The two kinds of sclerotia were planted at the same time in the same soil but in separate plates. The peeled sclerotia were obtained by peeling off the black layer by means of a razor blade as in series No. 6. Care was taken to select only those unpeeled sclerotia which were complete with both tail and head.

Observations were made 32 days after planting. The results are shown in Table No. 13.

Table No. 13 - Percentage of visible area of peeled and unpeeled sclerotia of C. purpurea attacked by micro-organisms in natural soil

TREATED

peeled

No.	Replication Number	1	2	3	4	5
1	1	100	100	100	100	80
2	2	100	100	100	100	80
3	3	100	100	100	100	100

unpeeled

No.	Replication Number	1	2	3	4	5
1	1	100	20	80	40	70
2	2	90	90	85	30	70
3	3	100	50	30	60	40

TREATED AND WASHED

peeled

No.	Replication Number	1	2	3	4	5
1	1	100	95	95	95	95
2	2	100	100	100	100	40
3	3	100	100	20	30	80

unpeeled

No.	Replication Number	1	2	3	4	5
1	1	20	80	50	50	40
2	2	0	20	40	30	80
3	3	100	90	20	40	30

UNTREATED

peeled

No.	Replication Number	1	2	3	4	5
1	1	0	0	0	80	40
2	2	0	0	0	0	20
3	3	30	35	20	40	20

unpeeled

No.	Replication Number	1	2	3	4	5
1	1	10	0	0	0	0
2	2	50	0	0	0	0
3	3	0	0	0	0	0

Average of percentages of visible area of peeled and unpeeled sclerotia of C. purpurea attacked by micro-organisms in each treatment

No.	Treatment	Peeled	Unpeeled
1	Treated	97.3	63.7
2	Treated and Washed	83.3	46.0
3	Untreated	19.0	4.0

Table No. 13 shows that:-

(1) The percentage of visible area attacked by micro-organisms in both peeled and unpeeled sclerotia was highest in treated and lowest in untreated sclerotia.

(2) The percentage of visible area attacked by micro-organisms was more in peeled sclerotia than in unpeeled sclerotia. (Fig. 11).

(3) All the peeled sclerotia were attacked by micro-organisms except 7 in the untreated group.

(4) All the unpeeled sclerotia were attacked by micro-organisms except 1 in the treated and washed group and 13 in the untreated group.

(5) The number of peeled sclerotia attacked by micro-organisms was greater than the number of unpeeled sclerotia so attacked.

Series 8 - Behavior of Peeled Sclerotia of S. sclerotiorum in Natural Soil

Experiment 1

In view of the results obtained in the experiment in which the cut surfaces of sclerotia were found to be more readily attacked

Fig. 11 Behavior of peeled and unpeeled sclerotia of Claviceps purpurea in natural soil

Upper row - Unpeeled sclerotia

Lower row - Peeled sclerotia

Dishes 1 - r - Treated and washed, treated and untreated

Upper row - Lubricated aspirators

Lower row - Vented aspirators

Diagram 1 - 1 - Thread and washer, threaded rod,

mutilated

than uninjured surfaces by soil micro-organisms, it was decided to investigate the behavior of peeled sclerotia of S. sclerotiorum in natural soil.

Black soil from the West Field of the University of Alberta was used in this study. The black outer layer of the sclerotia was removed, though not completely by means of a flat file and a hand grinder. This black layer is not uniform on the sclerotium nor is it readily separated from the other part so only its partial removal was possible. The same methods as in the previous experiments with C. purpurea were followed. The peeled sclerotia with and without treatment were simply planted on moist natural soil in deep Petri dishes and examined at intervals thereafter.

It was observed after about 6 weeks (43 days) following planting that some "black bodies" were formed around the planted sclerotia in all the treatments (Figure 12). It was also observed that the original planted sclerotia were degraded completely in all cases that is both treated and untreated. (Figure 13).

The "black bodies" which were formed around the original sclerotia were plated on solidified sterilized P.S.A. medium after surface sterilization with Perfex (diluted 10 times with sterilized distilled water) and washing with sterilized distilled water. Controls were made by plating in a similar manner, sclerotia obtained from a known artificial culture of S. sclerotiorum, and treating them in the same way as the "black bodies". The plates were kept at room temperature. It was found that the "black bodies" germinated to produce mycelium which formed new

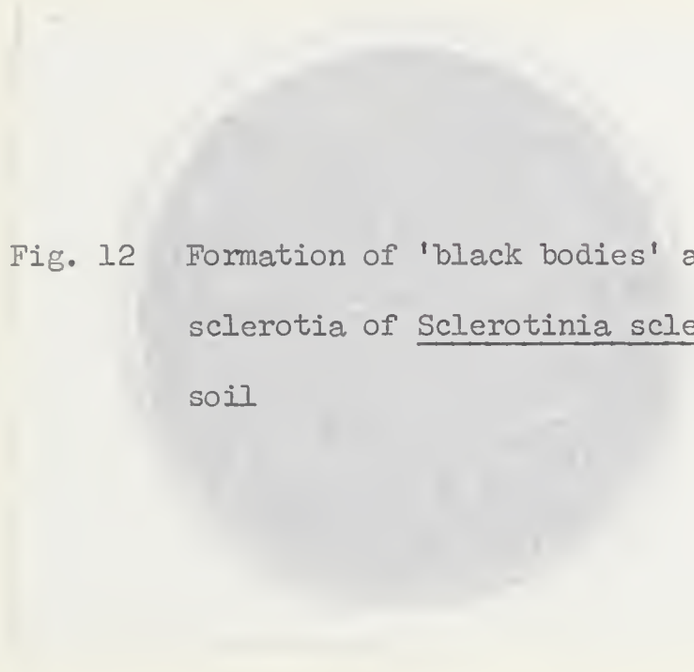


Fig. 12 Formation of 'black bodies' around the peeled sclerotia of Sclerotinia sclerotiorum in natural soil

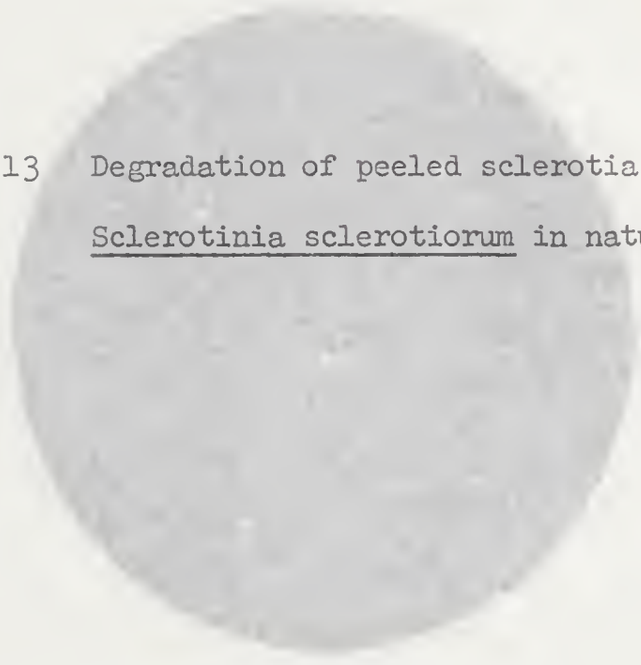


Fig. 13 Degradation of peeled sclerotia of *Sclerotinia sclerotiorum* in natural soil

Sclerotinia sclerotiorum in natural soil

Fig. 13 Deformation of hooked sclerotia of

sclerotia like those formed in the control plates (Figure 14). This proved that the black bodies were sclerotia of S. sclerotiorum. Therefore, the black bodies formed around peeled sclerotia of S. sclerotiorum were called "secondary" sclerotia. It was also observed that some black bodies were hollow inside. These did not necessarily grow and form new sclerotia as they were incompletely formed and matured. It was also observed with the aid of a microscope that the walls of such black bodies were designed like parenchyma cells, which is characteristic of sclerotia. Therefore, these hollow black bodies are here called "pseudo" sclerotia. Thus it may be said that the black bodies formed around the peeled sclerotia consist of a mixture of "secondary" and "pseudo" sclerotia.

It should be noted in contrast that no "pseudo" or "secondary" sclerotia were formed from peeled sclerotia of C. purpurea (Series 6) but the sclerotia were completely degraded after about 33 weeks (229 days) on natural soil especially in the treated group (Figure 13).

The formation of "pseudo" and "secondary" sclerotia of S. sclerotiorum in natural soil have not to the writer's knowledge been previously reported.

Series 9 - Behavior of Sclerotia of C. purpurea in Frozen Soil

Experiment 1

Some of the sclerotia which are produced either on spring rye or fall rye or on other grasses fall on the ground and overwinter there before they germinate. In cold countries like Canada overwintering

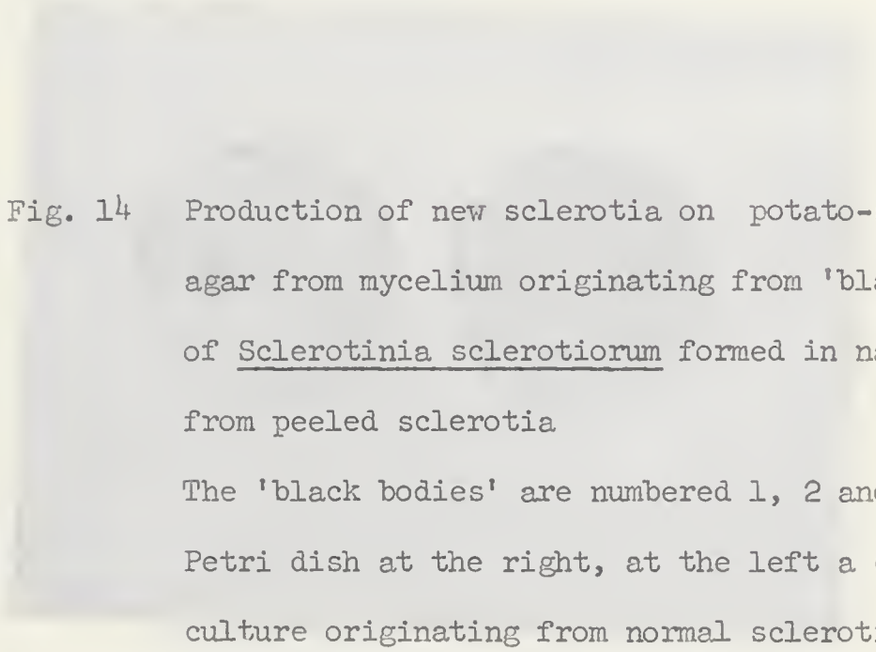
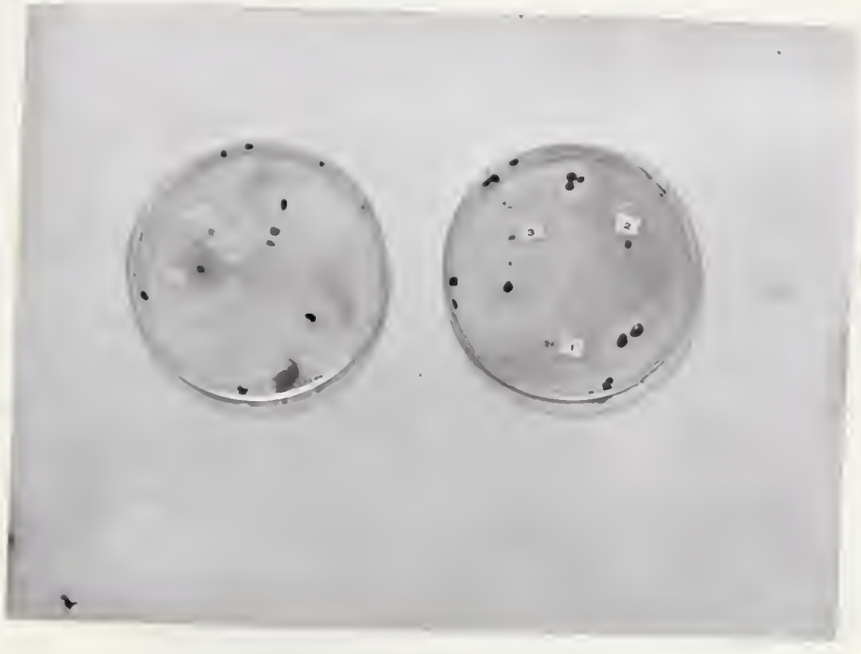


Fig. 14 Production of new sclerotia on potato-sucrose agar from mycelium originating from 'black bodies' of Sclerotinia sclerotiorum formed in natural soil from peeled sclerotia
The 'black bodies' are numbered 1, 2 and 3 in the Petri dish at the right, at the left a control culture originating from normal sclerotia is shown.



commonly occurs on or in frozen soil. Therefore attention has been given to study the behavior of sclerotia on soil frozen previous to its use.

Natural soil in a frozen state was collected in a sterilized container from a cultivated field in February 1959 when there was snow on the ground in the fields. It was stored in a refrigerator at $2^{\circ} \pm 2C^{\circ}$ for 22 days until it was added to Petri plates. This was done with care to avoid contamination from the air.

The procedure was the same as in experiments of series 1 except that 60 gm. of thawed soil were used per Petri dish. The dishes were kept in an incubator at $15^{\circ}C$. No extra moisture was added. None of the sclerotia germinated in this experiment. Observations on the percentage of the visible area of the sclerotia attacked by micro-organisms were made. The data taken about 12 weeks (85 days) after planting have been tabulated in Table No. 14.

Table No. 14 - Percentage of visible area of sclerotia of C. purpurea attacked by micro-organisms in frozen soil

TREATED

Observations

No.	Replication Number	1	2	3	4	5
1	1	100	100	95	90	25
2	2	100	100	70	20	40
3	3	90	100	60	5	20

TREATED AND WASHED

Observations

No.	Replication Number	1	2	3	4	5
1	1	100	10	10	15	25
2	2	90	45	50	80	20
3	3	100	100	30	20	25

UNTREATED

Observations

No.	Replication Number	1	2	3	4	5
1	1	0	0	0	0	15
2	2	0	0	0	0	0
3	3	20	0	0	0	35

Average of percentages of visible area of sclerotia of C. purpurea attacked by micro-organisms in each treatment

No.	Treatment	
1	Treated	67.7
2	Treated and Washed	48.0
3	Untreated	4.7

Table No. 14 shows that:-

(1) The percentage of visible area of the sclerotia attacked by micro-organisms was highest in the treated group and lowest in the untreated

group. (Figure 15).

(2) All the sclerotia were attacked by micro-organisms except 12 out of 15 in the untreated group.

Experiment 2

In another experiment ordinary soil from the West Field collected during the summer was mixed with snow and frozen by storing it in an incubator at -12°C for 31 days. Treated and untreated sclerotia of C. purpurea were planted in this soil immediately after it was thawed and moistened with sterile water.

Observations on the percentage of visible area of the sclerotia attacked by micro-organisms and on the germination of the sclerotia were made after 85 days and 100 days following planting. The results are recorded in Table 15 and Table 16.

Table No. 15 - Percentage of visible area of sclerotia of C. purpurea attacked by micro-organisms in natural soil after freezing it at -12°C.

TREATED

First Observation

No.	Replication Number	1	2	3	4	5
1	1	100	100	100	70	70
2	2	100	100	100	100	100
3	3	100	90	90	85	0
4	4	100	100	100	100	90
5	5	100	100	100	100	60
6	6	95	90	100	60	0

Second Observation

No.	Replication Number	1	2	3	4	5
1	1	100	100	100	100	100
2	2	100	100	100	100	100
3	3	100	100	100	90	85
4	4	100	100	100	100	100
5	5	100	100	100	100	100
6	6	100	100	95	50	00

TREATED AND WASHED

First Observation

No.	Replication Number	1	2	3	4	5
1	1	100	80	50	10	0
2	2	100	100	100	100	10
3	3	100	100	100	90	0
4	4	100	100	100	0	0
5	5	100	100	100	100	0
6	6	100	100	100	100	90

Second Observation

No.	Replication Number	1	2	3	4	5
1	1	100	90	80	80	0
2	2	100	100	100	100	80
3	3	100	100	100	90	0
4	4	100	100	100	40	20
5	5	100	100	100	100	10
6	6	100	100	100	100	100

UNTREATED

First Observation

No.	Replication Number	1	2	3	4	5
1	1	0	0	0	0	0
2	2	0	0	0	0	0
3	3	90	0	0	0	0
4	4	0	0	0	0	0
5	5	0	0	0	5	0
6	6	0	10	0	0	0

Second Observation

No.	Replication Number	1	2	3	4	5
1	1	0	0	0	0	0
2	2	0	0	0	0	0
3	3	100	0	0	0	0
4	4	0	0	0	0	0
5	5	0	0	0	0	0
6	6	20	50	0	0	0

Average of percentages of visible area of sclerotia of C. purpurea attacked by micro-organisms in each treatment

No.	Treatment	First Observation	Second Observation
1	Treated	83.6	94.0
2	Treated and Washed	77.3	83.0
3	Untreated	3.5	5.7

Table No. 15 shows that:-

(1) The percentage of visible area attacked by micro-organisms was highest in the treated group and lowest in the untreated group.

(2) There were 29 out of 30 sclerotia in treated group, 28 out of 30 in treated and washed group but only 3 out of 30 in the untreated group attacked by micro-organisms.

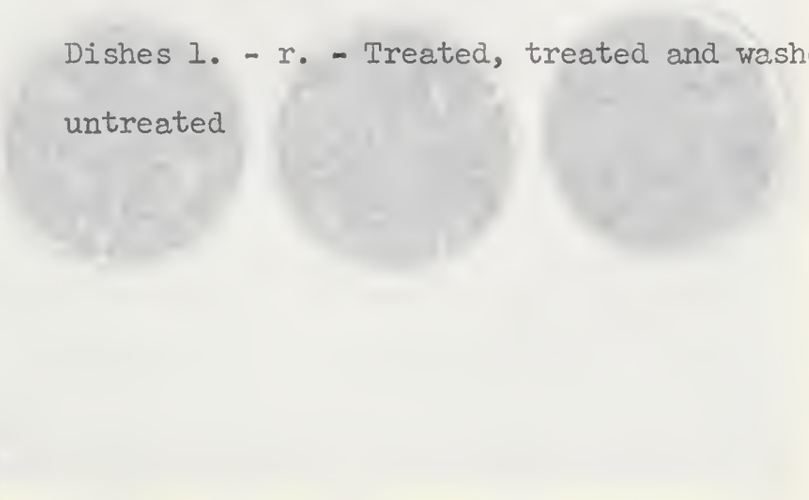


Fig. 15 Behavior of sclerotia of Claviceps purpurea in frozen soil after thawing

Dishes l. - r. - Treated, treated and washed, and untreated

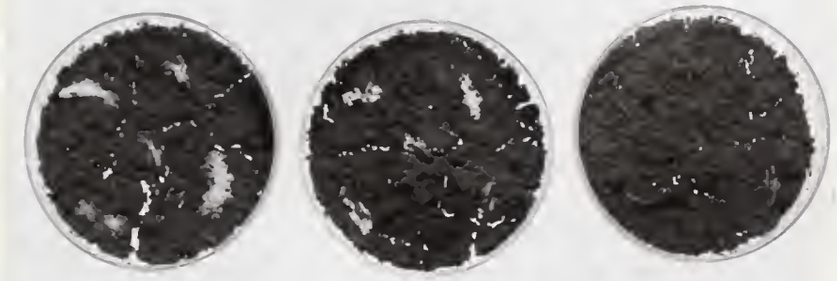


Table No. 16 - Germination of sclerotia of C. purpurea in natural soil after freezing at -12°C.

TREATED

First Observation

No.	Replication Number	1	2	3	4	5
1	1	-	-	-	-	-
2	2	-	-	-	-	-
3	3	-	-	-	-	+
4	4	-	-	-	-	-
5	5	-	-	-	-	-
6	6	-	-	-	-	+

+ Germinated - Not germinated

Second Observation

No.	Replication Number	1	2	3	4	5
1	1	-	-	-	-	-
2	2	-	-	-	-	-
3	3	-	-	-	+	-
4	4	-	-	-	-	-
5	5	-	-	-	-	-
6	6	-	-	-	-	+

+ Germinated - Not germinated

TREATED AND WASHED

First Observation

No.	Replication Number	1	2	3	4	5
1	1	-	-	-	-	-
2	2	-	-	-	-	-
3	3	-	-	-	-	+
4	4	-	-	-	+	+
5	5	-	-	-	-	+
6	6	-	-	-	-	-

+ Germinated - Not germinated

Second Observation

No.	Replication Number	1	2	3	4	5
1	1	-	-	-	-	-
2	2	-	-	-	-	-
3	3	-	-	-	-	+
4	4	-	-	-	+	+
5	5	-	-	-	-	+
6	6	-	-	-	-	-

+ Germinated - Not germinated

UNTREATED

First Observation

No.	Replication Number	1	2	3	4	5
1	1	-	-	-	-	+
2	2	-	-	-	-	+
3	3	-	-	-	-	+
4	4	-	-	-	-	+
5	5	-	-	-	-	+
6	6	-	-	+	+	+

+ Germinated - Not germinated

Second Observation

No.	Replication Number	1	2	3	4	5
1	1	-	-	+	+	+
2	2	-	-	-	+	+
3	3	-	-	-	+	+
4	4	-	-	+	+	+
5	5	-	-	+	+	+
6	6	+	+	+	+	+

+ Germinated - Not germinated

Summary of germination of sclerotia of C. purpurea in natural frozen soil

No.	Treatment	No. of Sclerotia Planted	First Observation	Second Observation
1	Treated	30	2	2
2	Treated and Washed	30	4	4
3	Untreated	30	8	18

Table No. 16 shows that:-

(1) Only 2 out of 30 sclerotia in the treated group, 4 out of 30 sclerotia in the treated and washed group and 18 out of 30 in the untreated group, germinated.

These two experiments show that frozen soil contains viable micro-organisms capable of attacking sclerotia of C. purpurea especially those treated with mercuric chloride. However only a small percentage of untreated sclerotia were attacked as compared with a large percentage in unfrozen soil in previous experiments. Moreover some germination of sclerotia in frozen soil occurred whereas little or none occurred in unfrozen soil in previous experiments. These results indicate a retardation of activity of the soil micro-organisms by freezing though it is realized that a comparison of results of different experiments is not justified. They do, however, suggest the desirability of further studies on this question.

Series 10 - Behavior of Sclerotia of S. sclerotiorum in Frozen Soil

Experiment 1

The sclerotia of S. sclerotiorum are commonly formed on and in the basal parts of host plants and are left on or in the soil when these parts decay. In cold countries like Canada, Russia, parts of Europe etc. the fungus is inactive throughout the winter but resumes activity in the spring and summer. During the winter the sclerotia are normally in contact with frozen soil and are not subjected to degradation by soil micro-organisms. When the soil warms up in the spring, however, soil micro-organisms become active and may attack the sclerotia. To determine if such freezing of the soil destroys its degrading power, sclerotia of S. sclerotiorum were planted in natural soil that had been frozen and thawed just before planting. Similar procedures to those of the first experiment of the last series with C. purpurea were followed and observations on the behavior of the sclerotia were made after 85 days. These are tabulated in Table No. 17.

Table No. 17 - Percentage of visible area of sclerotia of S. sclerotiorum attacked by micro-organisms in frozen soil

TREATED

Observation

No.	Replication Number	1	2	3	4	5	6	7	8	9
1	1	80	0	0	0	0	0	0	0	0
2	2	60	70	0	0	0	0	0	0	0
3	3	0	0	0	0	0	0	0	0	0

TREATED AND WASHED

Observation

No.	Replication Number	1	2	3	4	5	6	7	8	9
1	1	100	0	0	0	0	0	0	0	0
2	2	0	0	0	0	0	0	0	0	0
3	3	0	0	0	0	0	0	0	0	0

UNTREATED

Observation

No.	Replication Number	1	2	3	4	5	6	7	8	9
1	1	0	0	0	0	0	0	0	0	0
2	2	0	0	0	0	0	0	0	0	0
3	3	0	0	0	0	0	0	0	0	0

Average of percentages of visible area of sclerotia of S. sclerotiorum attacked by micro-organisms in each treatment

No.	Treatment	
1	Treated	7.8
2	Treated and Washed	3.7
3	Untreated	0.0

Table No. 17 shows that:-

(1) Only 3 out of 27 sclerotia of the treated group, 1 out of 27 of the treated and washed group were attacked by micro-organisms and none in the untreated group was attacked.

(2) The percentage of the visible area of the sclerotia attacked by micro-organisms was highest in the treated group and lowest in the untreated group.

The experiment shows that frozen soil contains viable micro-organisms capable of attacking sclerotia of S. sclerotiorum.

Although direct comparison was not made of the behavior of sclerotia in unfrozen and frozen soil there is some indication that the sclerotia were attacked more in unfrozen soil than in soil frozen and thawed just before planting. In both soils untreated sclerotia were not attacked whereas treated sclerotia were attacked. Thus it appears that treatment of sclerotia with mercuric chloride is of value in encouraging degradation in both frozen and unfrozen soil. It also would seem that the chances of sclerotia surviving are somewhat better in frozen soil than in unfrozen soil.

Series 11 - Time Required for Complete Degradation of Unpeeled Sclerotia of C. purpurea in Natural Soil

Experiment 1

Studies were begun in June 1959 to determine the approximate time required for complete degradation of normal unpeeled sclerotia of C. purpurea in natural soil. The procedure was the same as in the first

experiment of the first series. Treated, treated and washed and untreated sclerotia were planted in moist natural soil in Petri dishes and their positions were marked by sterilized straight pins.

It was observed after 6 weeks following planting that micro-organisms were attacking the sclerotia in all groups especially the treated and treated and washed ones. As a result the sclerotia were beginning to degrade. However five sclerotia out of 15 in the untreated group were found germinating and producing the perfect stage of the pathogen.

The plates were examined every month following planting and watered with distilled water when necessary. Regular observations were recorded after 9 months following planting. The number of completely degraded sclerotia was noted.

The observations made in the 13th month (the last month of observation) are recorded in Table No. 18. (Figure 16).

Table No. 18 - Behavior of unpeeled sclerotia of C. purpurea regarding their complete degradation as a result of attack of micro-organisms in each treatment after 13 months on natural soil

TREATED

No.	Replication Number	1	2	3	4	5
1	1	-	-	-	-	-
2	2	-	-	-	-	-
3	3	-	-	-	-	-

+ Completely degraded - Incompletely degraded

TREATED AND WASHED

No.	Replication Number	1	2	3	4	5
1	1	-	-	-	-	-
2	2	-	-	-	-	-
3	3	-	-	-	-	+

+ Completely degraded - Incompletely degraded

UNTREATED

No.	Replication Number	1	2	3	4	5
1	1	-	-	-	-	-
2	2	-	-	-	-	-
3	3	-	-	-	-	-

+ Completely degraded - Incompletely degraded or not degraded (4 only of untreated)

Table No. 18 shows that:-

(1) One sclerotium of the treated and washed group was completely degraded as compared with none in the untreated group.

(2) One third of the untreated sclerotia had germinated to produce the perfect stage whereas none of the treated group had germinated.

Under the conditions of the experiment complete degradation of the sclerotia of C. purpurea was a slow process. The fact that none was observed among the untreated ones after 13 months and that some of them had germinated where one of the treated ones had been completely degraded

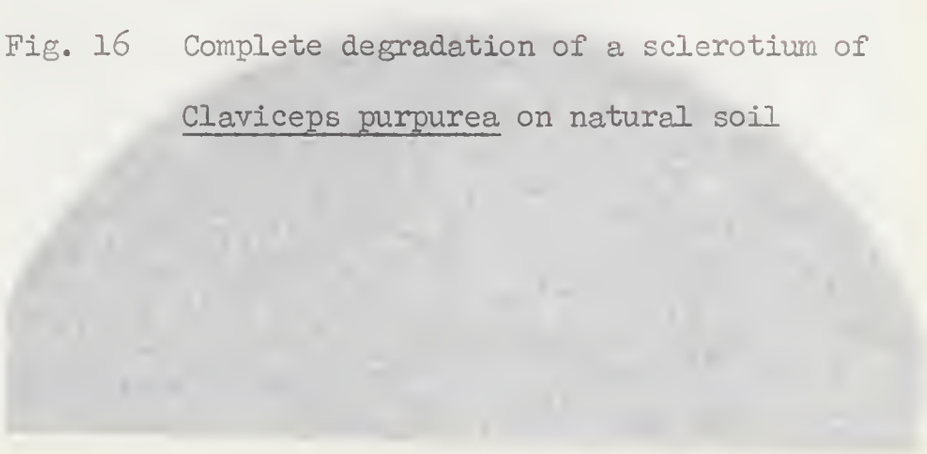


Fig. 16 Complete degradation of a sclerotium of *Claviceps purpurea* on natural soil

Fig. 16 Complete degradation of a sclerotium of *Clavicaps purpurea* on natural soil

in that time and none of them had germinated suggests that treatment with mercuric chloride tends to predispose the sclerotia of C. purpurea to degradation in natural soil. Moreover the results of other experiments showing that such treatment encourages soil micro-organisms to attack the sclerotia suggest that they may be responsible for degradation. They are probably responsible for both incomplete and complete degradation. It should be noted though that the former may be quite sufficient to prevent germination but failure to germinate alone does not necessarily imply microbial degradation.

Series 12 - Time Required for Complete Degradation of Peeled Sclerotia of C. purpurea in Natural Soil

Experiment 1

The experimental material of series 6 in which peeled sclerotia of each treated group were planted on natural soil in Petri dishes was further watched for the complete degradation of the sclerotia.

Complete degradation was considered to have occurred when the body of the sclerotium had completely disintegrated leaving only a depression in the soil marking its location.

It was observed after 5 months that no sclerotium in any group had been degraded completely. In 6 months one sclerotium in the treated group had degraded completely. In 7 months 2 sclerotia of the same group in the same plate were degraded completely. (Figure 17). The observations made after 7 months are recorded in Table No. 19. They show the same trend as those for unpeeled sclerotia. While a direct comparison is not justified, a more rapid degradation of peeled sclerotia is indicated.

Fig. 17 Behavior of peeled sclerotia of Claviceps purpurea in natural soil
Two treated sclerotia have been completely degraded after 7 months

Table No. 19 - Behavior of peeled sclerotia of C. purpurea regarding their complete degradation as a result of attack of micro-organisms in each treatment after 9 months on natural soil

TREATED

No.	Replication Number	1	2	3	4	5
1	1	-	-	-	-	-
2	2	-	-	-	-	+
3	3	-	-	-	-	+

\+ Completely degraded - Incompletely degraded

TREATED AND WASHED

No.	Replication Number	1	2	3	4	5
1	1	-	-	-	-	-
2	2	-	-	-	-	-
3	3	-	-	-	-	-

\+ Completely degraded - Incompletely degraded

UNTREATED

No.	Replication Number	1	2	3	4	5
1	1	-	-	-	-	-
2	2	-	-	-	-	-
3	3	-	-	-	-	-

\+ Completely degraded - Incompletely degraded or not degraded (2 only of untreated)

P A R T III

ISOLATION AND IDENTIFICATION OF FUNGI

It has been noted in Part II that the sclerotia of C. purpurea and S. sclerotiorum were attacked by other micro-organisms especially when they were treated with mercuric chloride and sown in natural soil. It was of interest to identify at least those micro-organisms that appeared most commonly on the sclerotia and since fungi appeared to be the dominant ones attention was confined to them.

Section (a) Isolation of Fungi

Isolations were made of fungi found attacking the sclerotia of C. purpurea and S. sclerotiorum planted in experiment one of series 1, 2, 9 and 10 in natural and frozen soil using the following procedure:-

A bit of mycelium with attached spores if any, of the fungus to be isolated was transferred aseptically with a sterilized needle from the attacked sclerotium to a sterilized plate of plain water agar (2%). Two transfers from opposite sides of a typical colony were then made to sterilized plates of acid potato sucrose agar. These isolates were allowed to grow for 3 - 4 days at room temperature and then transferred to test tube slants of P.D.A. Table No. 20 shows the number of isolates of each fungus obtained from the sclerotia of each pathogen.

Table No. 20 - Number of fungal isolates isolated from sclerotia of C. purpurea and S. sclerotiorum which were attacked by micro-organisms in natural and frozen soil

No.	Soil	Sclerotia	Treated	Treated and Washed	Untreated	Total
1	Natural Soil	C. purpurea	145	145	110	400
		S. sclerotiorum	50	50	-	100
2	Frozen Soil	C. purpurea	133	66	33	232
		S. sclerotiorum	35		-	35

Section (b) Identification of Fungi Attacking Sclerotia of C. purpurea and S. sclerotiorum in Natural and Frozen Soil

The technique employed in identifying the fungi isolated from the sclerotia of C. purpurea and S. sclerotiorum planted in natural soil is described below:-

Sterilized 2 x 9 cm. Petri plates were poured with water agar (2%), and thus served as moist chambers. Approximately 15 ml. of agar were used per plate. Oven sterilized microscope slides were dipped in either sterilized corn meal agar or potato dextrose agar and mounted aseptically on the solidified water agar in the Petri dishes. Two slides each of potato dextrose agar and corn meal agar were mounted in each Petri dish. A bit of mycelium of each isolate was transferred aseptically to each slide. The fungus was grown on the slides for 2 - 3 days when it was examined microscopically and identified to genus.

Table No. 21 - Fungi identified which were attacking the sclerotia of C. purpurea and S. sclerotiorum in frozen and natural soil

NATURAL SOIL								
			C. PURPUREA				S. SCLEROTIORUM	
No.	Treatment	No. of Isolates	FUNGI IDENTIFIED	FREQUENCY	Treatment	No. of Isolates	FUNGI IDENTIFIED	FREQUENCY
1	Treated	145	Penicillium	4	Treated	50	Trichoderma	14
			Gliocladium and Fusarium	3			Gliocladium	11
			Gliocladium and Penicillium	136			Gliocladium and Penicillium	24
			Penicillium and Fusarium	2			Fusarium and Gliocladium	1
2	Treated and Washed	145	Gliocladium and Penicillium	100	Treated and Washed	50	Trichoderma	3
			Gliocladium	40			Gliocladium	2
			Penicillium	3			Gliocladium and Penicillium	42
			Gliocladium and Fusarium	2			Fusarium and Gliocladium	3
3	Untreated	110	Gliocladium	9	Untreated	-	-	-
			Gliocladium and Penicillium	100				
			Gliocladium and Fusarium	1				

Table 21 Continued

NATURAL FROZEN SOIL								
			C. PURPUREA				S.SCLEROTIORUM	
No.	Treatment	No. of Isolates	FUNGI IDENTIFIED	FREQUENCY	Treatment	No. of Isolates	FUNGI IDENTIFIED	FREQUENCY
1	Treated	133	Fusarium	1	Treated and Treated and Washed	35	Trichoderma	34
			Gliocladium and Penicillium	132			Gliocladium and Trichoderma	1
2	Treated and Washed	66	Gliocladium	63				
			Aspergillus	1				
			Fusarium	1				
			Penicillium	1				
3	Untreated	33	Gliocladium	1			-	-
			Gliocladium and Penicillium	31				
			Gliocladium, Penicillium and Fusarium	1				

Table 21 shows that:-

(1) Gliocladium sp. attacked the sclerotia of C. purpurea and S. sclerotiorum frequently in both frozen and unfrozen natural soil.

(2) Other fungi less frequently isolated were Penicillium, Fusarium and Trichoderma.

(3) Gliocladium often occurred in a mixture with Penicillium.

(4) The fungi Gliocladium, Penicillium, Fusarium, Trichoderma survived in frozen soil.

(5) Untreated sclerotia of S. sclerotiorum remained free from visible contaminating fungi in both unfrozen and frozen natural soil.

P A R T IV

EFFECT OF GLIOCLADIUM SP. ON SCLEROTIA

Table 21 shows that Gliocladium sp. was the most commonly found fungus attacking the sclerotia of two phytopathogenic fungi, C. purpurea and S. sclerotiorum. It occurred very frequently on sclerotia of the former. Therefore studies were undertaken to determine the pathogenic effect of Gliocladium on the sclerotia of C. purpurea and S. sclerotiorum.

Section (a) Preparation of Sclerotia for Pathogenicity Tests

In order to test the pathogenicity of one fungus on another it is important that the host fungus be free from other micro-organisms. The sclerotia of C. purpurea collected in the field are usually contaminated with other micro-organisms. Honey dew of the ergot fungus is often found on the surfaces of the sclerotia. This may encourage the growth of other micro-organisms or cause them to adhere to the sclerotia. The contamination of sclerotia of S. sclerotiorum produced on artificial media is much less likely but some might occur when they are being harvested or during storage. Therefore the following methods were adopted to get uncontaminated sclerotia of both fungi.

Sclerotia of S. sclerotiorum

Sclerotia of S. sclerotiorum produced on an artificial medium in Petri dishes were not collected immediately but the mycelium was allowed to grow for one month at room temperature. The dishes were

subsequently stored at 5°C. for 2 months before the sclerotia were used in experiments. The purpose of storing the dishes was to dry the medium so that the sclerotia would come off easily.

Sclerotia of C. purpurea

In preliminary tests 99% of the sclerotia of C. purpurea available for experimental work were found contaminated when planted aseptically on sterilized P.D.A. in Petri dishes (2 x 9 cm.) and incubated at room temperature for two weeks. In order to free the sclerotia from micro-organisms, they were subjected to hot water treatment.

In these treatments the sclerotia were soaked 1 - 6 hours in tap water and then immersed for a certain time in a hot water bath maintained at a definite temperature, and then dried at room temperature. In the hot water bath part of the treatment the sclerotia were placed in cheese-cloth bags and immersed for periods of 5 - 30 minutes in water held at each of the following temperatures; 104°F. (40°C.), 112°F. (44.4°C.), 122°F. (50°C.), 131°F. (55°C.), and 140°F. (60°C.) respectively. After drying they were stored in paper bags at approximately 32°F (0°C.).

The treated sclerotia were screened by plating them on solidified potato dextrose agar in Petri dishes (2 x 9 cm.) each containing 15 ml. of the sterilized medium. Before the sclerotia as treated above, were plated they were dipped in 50% ethyl alcohol and flamed in a Bunsen flame. The plates were incubated at room temperature for 3 weeks. It was

observed that sclerotia treated with hot water even at the highest temperature remained viable. This was proved by germinating them successfully to produce the perfect stage in soil and by inducing them to form the conidial or imperfect stage on agar.

The uncontaminated sclerotia that is those with no growths of other organisms around them on the agar were picked up aseptically and stored in sterilized Petri dishes in a refrigerator. The results of the hot water treatments of sclerotia of C. purpurea are shown in Table No. 22.

Table No. 22 shows that sclerotia of C. purpurea may survive hot water treatments at 104°F, 112°F., 122°F., 131°F., and 140°F. for 5 - 30 minutes following soaking periods of 1 - 6 hours.

A summary of the hot water treatments of the sclerotia of C. purpurea is given in Table No. 23.

Table No. 23 - Summarized results of hot water treatment of sclerotia of C. purpurea

No.	Temperature of Treatment	No. of Sclerotia Plated	Contaminated	Uncontaminated	% Uncontaminated
1	104°F	349	293	56	16.04
2	112°F	356	266	90	25.28
3	122°F	360	270	90	25.00
4	131°F	355	155	200	56.34
5	140°F	351	103	248	70.66

Table No. 22 - Results of hot water treatment of sclerotia of C. purpurea

TEMPERATURE 104°F.

No.	Soaking Period in hours	Treat time in minutes	Number of Sclerotia Plated*	Uncontaminated Non-Germinated	Uncontaminated Germinated	Contaminated
1	1	5	9(2)	-	2	7
2	1	10	9(2)	-	1	8
3	1	15	10(2)	2	1	7
4	1	20	10(2)	-	2	8
5	1	25	10(2)	-	1	9
6	1	30	10(2)	-	2	8
7	2	5	10(2)	-	-	10
8	2	10	8(2)	-	-	8
9	2	15	9(2)	1	1	7
10	2	20	10(2)	-	-	10
11	2	25	10(2)	-	-	9
12	2	30	9(2)	-	1	8
13	3	5	10(2)	1	-	9
14	3	10	10(2)	-	3	7
15	3	15	10(2)	-	1	9
16	3	20	10(2)	-	-	10
17	3	25	10(2)	-	1	9
18	3	30	10(2)	-	1	9
19	4	5	10(2)	-	1	9
20	4	10	10(2)	-	4	6
21	4	15	10(2)	-	2	8
22	4	20	10(2)	-	-	10
23	4	25	10(2)	-	-	10
24	4	30	10(2)	-	-	10
25	5	5	10(2)	-	-	10
26	5	10	9(2)	-	3	6
27	5	15	9(2)	1	-	8
28	5	20	10(2)	-	3	7
29	5	25	10(2)	-	2	8
30	5	30	9(2)	-	-	9
31	6	5	10(2)	-	4	6
32	6	10	10(2)	-	5	5
33	6	15	10(2)	1	4	5
34	6	20	9(2)	-	-	9
35	6	25	10(2)	-	1	9
36	6	30	9(2)	-	3	6
		TOTAL	349	6	50	293

* The figure in brackets shows the number of Petri dishes used for plating the sclerotia.

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Table 22 Continued

TEMPERATURE 112°F.

No.	Soaking Period in hours	Treat time in minutes	Number of Sclerotia			
				Uncontaminated		Contaminated
			Plated*	Non-Germinated	Germinated	
1	1	5	10(2)	-	-	10
2	1	10	10(2)	1	1	8
3	1	15	10(2)	3	1	6
4	1	20	10(2)	1	0	9
5	1	25	10(2)	2	0	8
6	1	30	8(2)	2	0	6
7	2	5	10(2)	4	0	6
8	2	10	10(2)	6	0	4
9	2	15	10(2)	4	1	5
10	2	20	8(2)	1	1	6
11	2	25	10(2)	4	1	5
12	2	30	10(2)	4	2	4
13	3	5	10(2)	2	1	7
14	3	10	10(2)	3	1	6
15	3	15	10(2)	2	0	8
16	3	20	10(2)	3	0	7
17	3	25	10(2)	2	1	7
18	3	30	10(2)	4	1	5
19	4	5	10(2)	1	0	9
20	4	10	10(2)	2	1	7
21	4	15	10(2)	1	0	9
22	4	20	10(2)	1	0	9
23	4	25	10(2)	3	1	6
24	4	30	10(2)	0	0	10
25	5	5	10(2)	1	3	6
26	5	10	10(2)	0	2	8
27	5	15	10(2)	0	1	9
28	5	20	10(2)	0	0	10
29	5	25	10(2)	3	0	7
30	5	30	10(2)	1	0	9
31	6	5	10(2)	3	0	7
32	6	10	10(2)	1	0	9
33	6	15	10(2)	2	0	8
34	6	20	10(2)	2	0	8
35	6	25	10(2)	0	0	10
36	6	30	10(2)	1	1	8
	TOTAL		356	70	20	266

* The figure in brackets shows the number of Petri dishes used for plating the sclerotia.

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Table 22 Continued

TEMPERATURE 122°F.

No.	Soaking Period in hours	Treat time in minutes	Number of Sclerotia			
			Plated*	Uncontaminated Non-Germinated	Uncontaminated Germinated	Contaminated
1	1	5	10(2)	2	0	8
2	1	10	10(2)	0	2	8
3	1	15	10(2)	0	2	8
4	1	20	10(2)	1	0	9
5	1	25	10(2)	3	4	3
6	1	30	10(2)	5	2	3
7	2	5	10(2)	1	0	9
8	2	10	10(2)	3	1	6
9	2	15	10(2)	4	0	6
10	2	20	10(2)	5	0	5
11	2	25	10(2)	1	4	5
12	2	30	10(2)	3	2	5
13	3	5	10(2)	0	0	10
14	3	10	10(2)	0	0	10
15	3	15	10(2)	6	0	4
16	3	20	10(2)	0	0	10
17	3	25	10(2)	0	0	10
18	3	30	10(2)	4	0	6
19	4	5	10(2)	0	0	10
20	4	10	10(2)	1	0	9
21	4	15	10(2)	1	0	9
22	4	20	10(2)	1	0	9
23	4	25	10(2)	1	0	9
24	4	30	10(2)	1	0	9
25	5	5	10(2)	0	0	10
26	5	10	10(2)	1	0	9
27	5	15	10(2)	2	0	8
28	5	20	10(2)	4	0	6
29	5	25	10(2)	1	0	9
30	5	30	10(2)	3	0	7
31	6	5	10(2)	1	0	9
32	6	10	10(2)	2	0	8
33	6	15	10(2)	0	0	10
34	6	20	10(2)	7	0	3
35	6	25	10(2)	4	0	6
36	6	30	10(2)	5	0	5
	TOTAL		360	73	17	270

* The figure in brackets shows the number of Petri dishes used for plating the sclerotia.

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Table 22 Continued

TEMPERATURE 131°F.

No.	Soaking Period in hours	Treat time in minutes	Number of Sclerotia			
			Plated*	Uncontaminated Non-Germinated	Uncontaminated Germinated	Contaminated
1	1	5	10(2)	0	2	8
2	1	10	10(2)	0	6	4
3	1	15	10(2)	2	4	4
4	1	20	10(2)	5	0	5
5	1	25	8(2)	1	1	6
6	1	30	10(2)	5	1	4
7	2	5	10(2)	3	5	2
8	2	10	10(2)	5	2	3
9	2	15	10(2)	4	0	6
10	2	20	10(2)	6	2	2
11	2	25	10(2)	5	0	5
12	2	30	10(2)	3	1	6
13	3	5	9(2)	1	1	7
14	3	10	10(2)	4	0	6
15	3	15	10(2)	6	1	3
16	3	20	10(2)	3	1	6
17	3	25	10(2)	5	0	5
18	3	30	10(2)	7	0	3
19	4	5	10(2)	3	2	5
20	4	10	8(2)	8	0	0
21	4	15	10(2)	8	1	1
22	4	20	10(2)	4	1	5
23	4	25	10(2)	3	0	7
24	4	30	10(2)	2	0	8
25	5	5	10(2)	1	1	8
26	5	10	10(2)	9	0	1
27	5	15	10(2)	5	0	5
28	5	20	10(2)	1	1	8
29	5	25	10(2)	1	1	8
30	5	30	10(2)	10	0	0
31	6	5	10(2)	7	0	3
32	6	10	10(2)	8	0	2
33	6	15	10(2)	6	1	3
34	6	20	10(2)	7	0	3
35	6	25	10(2)	7	0	3
36	6	30	10(2)	5	0	5
	TOTAL		355	166	34	155

* The figure in brackets shows the number of Petri dishes used for plating the sclerotia.

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Table 22 Continued

TEMPERATURE 140°F.

No.	Soaking Period in hours	Treat time in minutes	Number of Sclerotia			
			Plated*	Uncontaminated Non-Germinated	Uncontaminated Germinated	Contaminated
1	1	5	10(2)	1	1	8
2	1	10	6(2)	1	1	4
3	1	15	9(2)	2	2	5
4	1	20	10(2)	3	1	6
5	1	25	10(2)	3	1	6
6	1	30	10(2)	8	0	2
7	2	5	10(2)	3	0	7
8	2	10	10(2)	5	2	3
9	2	15	8(2)	1	3	4
10	2	20	10(2)	7	1	2
11	2	25	10(2)	5	1	4
12	2	30	10(2)	10	0	0
13	3	5	10(2)	7	0	3
14	3	10	10(2)	6	1	3
15	3	15	10(2)	7	0	3
16	3	20	10(2)	9	1	0
17	3	25	10(2)	7	2	1
18	3	30	10(2)	10	0	0
19	4	5	10(2)	8	0	2
20	4	10	10(2)	9	0	1
21	4	15	10(2)	9	0	1
22	4	20	10(2)	9	0	1
23	4	25	10(2)	9	0	1
24	4	30	10(2)	3	0	7
25	5	5	10(2)	5	2	3
26	5	10	10(2)	3	2	5
27	5	15	10(2)	4	0	6
28	5	20	10(2)	9	0	1
29	5	25	10(2)	6	3	1
30	5	30	10(2)	8	2	0
31	6	5	10(2)	10	0	0
32	6	10	10(2)	9	1	0
33	6	15	10(2)	7	0	3
34	6	20	8(2)	8	0	0
35	6	25	10(2)	8	0	2
36	6	30	10(2)	2	0	8
		TOTAL	351	221	27	103

* The figure in brackets shows the number of Petri dishes used for plating the sclerotia.

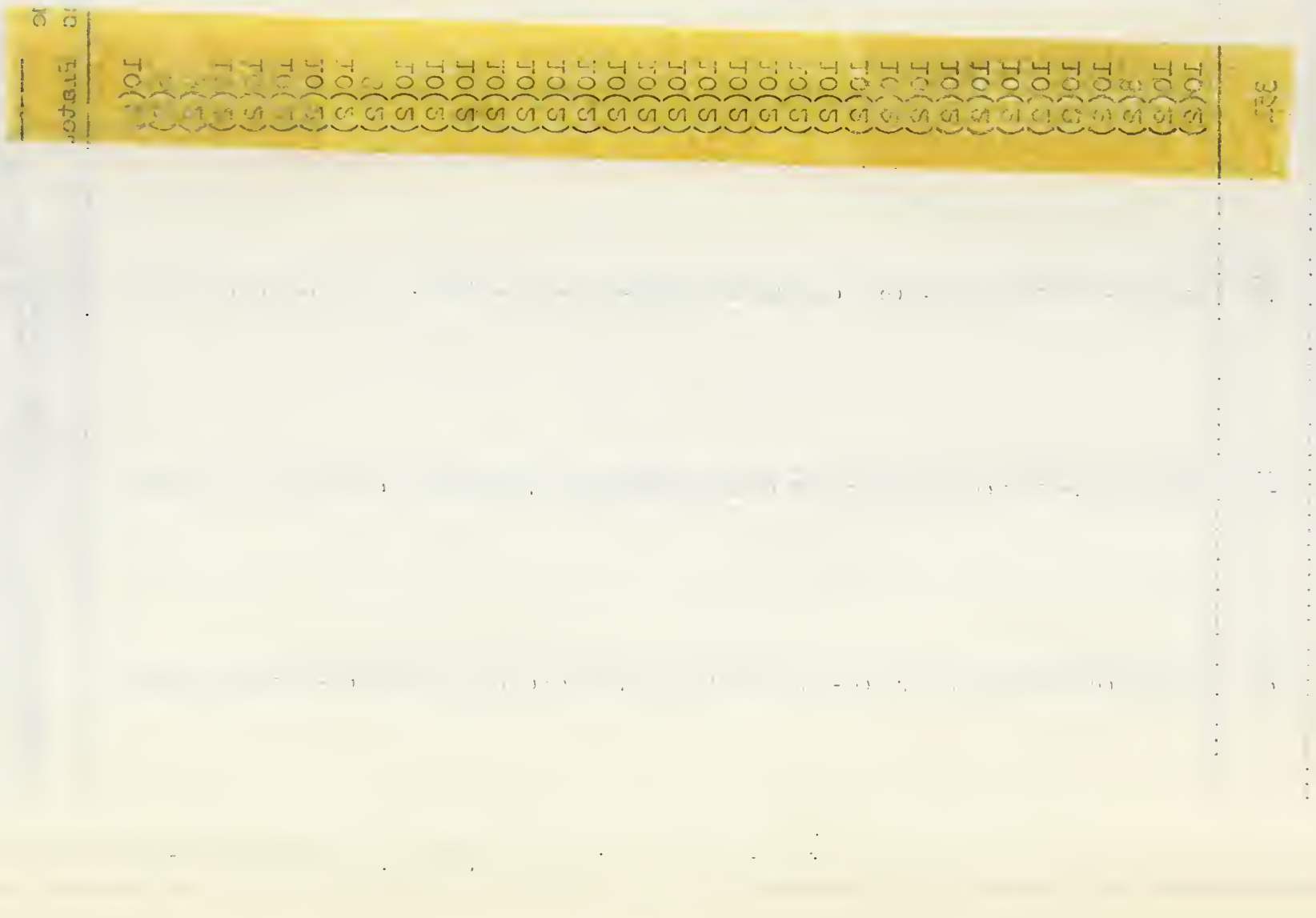
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Table No. 23 shows that uncontaminated sclerotia can be obtained by treatment with hot water. The best temperature for getting uncontaminated sclerotia was found to be 140°F. As contaminants of sclerotia are not single species of micro-organisms and since all sclerotia are not contaminated by a definite group of micro-organisms having the same thermal death points, no definite conclusion can be drawn as to the best temperature for all sclerotia of C. purpurea.

Section (b) Effect of Gliocladium sp. on sclerotia

As it is evident from Table No. 21 that Gliocladium sp. frequently attacks the sclerotia of both fungi, C. purpurea and S. sclerotiorum, especially the former, the effect of Gliocladium on sclerotia of both fungi was investigated.

Uncontaminated and ungerminated sclerotia of C. purpurea obtained as a result of hot water treatment (see Table 22) and sclerotia of S. sclerotiorum which were produced according to the method described earlier were used.

An isolate of Gliocladium sp. obtained from treated and washed sclerotia of C. purpurea planted in natural soil was used to inoculate sclerotia of both fungi. As this isolate of Gliocladium was at first found to be mixed with a Penicillium sp. it was necessary to separate it from the latter. This was readily accomplished by atomizing a spore suspension of the mixture onto sterilized solidified P.S.A. in Petri plates and incubating them at 25°C. (9) for 2 - 3 days. Separate Gliocladium colonies developed and a transfers were made from typical ones to P.S.A. test tube slants. The fungus produces a characteristic

yellow color which helps in recognizing it. After 7 days at 25°C. the purified fungus was sown on sterilized solidified P.S.A. in Petri dishes. The fungus was sown at 3 places in each dish. The plates were incubated for 15 days at 25°C. (9)

The effect of Gliocladium on treated, treated and washed and untreated sclerotia of both fungi was studied. Uncontaminated sclerotia were selected and those to be treated were immersed in an aqueous solution of $HgCl_2$ (1:000) for one minute. Deep Petri dishes were filled with soil and moistened as has been described in Part II. The dishes and contents were sterilized at 15 lbs. pressure for 3 hours in an autoclave. The sclerotia were inoculated in batches of 30 for each treatment by dipping them in 15 ml. of a spore suspension of Gliocladium sp. For each group (treatment) of sclerotia of both fungi, separate spore suspensions was used. The spores from one Petri dish were used in making a 15 ml. suspension in sterilized distilled water. In all 6 plates of fungus culture were used. The sclerotia were planted, with care to avoid contamination, in the Petri dishes of sterilized soil described above. Five sclerotia were planted in each Petri dish and their locations were indicated by metal pins (sterilized by dipping in 95% alcohol and flaming in a Bunsen flame). Controls consisted of plantings of uninoculated sclerotia of each treatment in sterilized soil plates. All plates were incubated at 15°C.

Observations were made on the percentage of visible area of sclerotia attacked by Gliocladium, on the germination of the sclerotia and on the degree of softening of the sclerotia. A sterilized needle.

was used to determine the degree of softness. In the present experiment only germination resulting in the development of the perfect stage of the pathogen in question was recorded as germination. The first observation was made about 10 weeks following inoculation and the second observation after about 12 weeks. The results are shown in Table No. 22. It was observed that no sclerotia of either fungus attacked by Gliocladium germinated as a result in any treatment.

Table No. 24 - Percentage of visible area of sclerotia of C. purpurea and S. sclerotiorum attacked by Gliocladium sp. and degrees of softness of sclerotia*

SCLEROTIA OF C. PURPUREA

TREATED

First Observation

Treated

No.	Replication Number	1	2	3	4	5
1	1	100 (1)	100(1)	100(1)	100(2)	100 (2)
2	2	100 (1)	100 (1)	100 (1)	100 (1)	90 (2)
3	3	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)
4	4	100 (1)	100 (1)	100 (1)	90 (1)	90 (2)
5	5	100 (1)	100 (1)	100 (1)	100 (1)	90 (1)
6	6	100 (1)	100 (1)	100 (1)	100 (1)	100 (2)

* The figure in parenthesis shows the degree of softness.

Degree of Softness

Hard - Needle fails to penetrate the sclerotium. (1)

Medium - Needle penetrates the sclerotium by force. (2)

Soft - Sclerotium keeps its shape. Needle penetrates easily. (3)

Mushy - Sclerotia very soft without any shape. (4)

Control

No.	Replication Number	1	2	3	4	5
1	1	+	-	-	-	-
2	2	+	-	-	-	-
3	3	+	-	-	-	-
4	4	+	-	+	+	-
5	5	+	-	-	-	-
6	6	+	-	-	-	-

+ Germinated - Not germinated

Second Observation

Treated

No.	Replication Number	1	2	3	4	5
1	1	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)
2	2	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)
3	3	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)
4	4	100 (2)	100 (2)	100 (2)	100 (2)	100 (3)
5	5	100 (3)	100 (2)	100 (2)	100 (2)	100 (2)
6	6	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)

Control

No.	Replication Number	1	2	3	4	5
1	1	+	-	-	-	-
2	2	+	-	-	-	-
3	3	+	-	-	-	-
4	4	+	+	+	+	-
5	5	+	-	-	-	-
6	6	+	-	-	-	-

+ Germinated - Not germinated

TREATED AND WASHED

First Observation

Treated and Washed

No.	Replication Number	1	2	3	4	5
1	1	100 (2)	100 (2)	100 (2)	100 (2)	100 (1)
2	2	100 (2)	100 (2)	100 (1)	100 (2)	100 (1)
3	3	100 (2)	100 (1)	100 (2)	100 (1)	100 (1)
4	4	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)
5	5	100 (2)	100 (2)	100 (1)	100 (1)	100 (1)
6	6	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)

Control

No.	Replication Number	1	2	3	4	5
1	1	+	+	-	-	-
2	2	+	+	-	-	-
3	3	+	-	-	-	-
4	4	+	+	-	-	-
5	5	+	+	-	-	-
6	6	+	+	-	-	-

+ Germinated - Not germinated

Second Observation

Treated and Washed

No.	Replication Number	1	2	3	4	5
1	1	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)
2	2	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)
3	3	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)
4	4	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)
5	5	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)
6	6	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)

Control

No.	Replication Number	1	2	3	4	5
1	1	+	+	-	-	-
2	2	+	+	-	-	-
3	3	+	+	-	-	-
4	4	+	+	-	-	-
5	5	+	+	-	-	-
6	6	+	+	-	-	-

+ Germinated - Not germinated

UNTREATED

First Observation

Untreated

No.	Replication Number	1	2	3	4	5
1	1	100 (1)	100 (1)	100 (1)	100 (1)	100 (2)
2	2	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)
3	3	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)
4	4	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)
5	5	100 (2)	100 (1)	100 (1)	100 (1)	100 (1)
6	6	100 (2)	100 (2)	100 (2)	100 (1)	100 (1)

Control

No.	Replication Number	1	2	3	4	5
1	1	-	-	-	-	-
2	2	-	-	-	-	-
3	3	-	-	-	-	-
4	4	+	+	-	-	-
5	5	-	-	-	-	-
6	6	+	+	-	-	-

\+ Germinated - Not germinated

Second Observation

Untreated

No.	Replication Number	1	2	3	4	5
1	1	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)
2	2	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)
3	3	100 (2)	100 (1)	100 (2)	100 (2)	100 (2)
4	4	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)
5	5	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)
6	6	100 (2)	100 (1)	100 (2)	100 (2)	100 (2)

Control

No.	Replication Number	1	2	3	4	5
1	1	+	-	-	-	-
2	2	-	-	-	-	-
3	3	-	-	-	-	-
4	4	+	+	-	-	-
5	5	-	-	-	-	-
6	6	+	+	-	-	-

+ Germinated - Not germinated

Average of percentages of visible area of sclerotia of C. purpurea attacked by Gliocladium sp. in each treatment

No.	Treatment	First Observation	Second Observation
1	Treated	98.7	100.0
2	Treated and Washed	100.0	100.0
3	Untreated	100.0	100.0

Average of degrees of softness of sclerotia of C. purpurea attacked by Gliocladium sp. in each treatment

No.	Treatment	First Observation	Second Observation
1	Treated	1.2	2.1
2	Treated and Washed	1.4	2.0
3	Untreated	1.2	1.9

SCLEROTIA OF S. SCLEROTIORUM

TREATED

First Observation

Treated

No.	Replication Number	1	2	3	4	5
1	1	100 (1)	100 (3)	100 (3)	100 (3)	100 (3)
2	2	100 (1)	100 (2)	100 (2)	100 (2)	100 (3)
3	3	100 (1)	100 (1)	100 (1)	100 (2)	100 (3)
4	4	100 (1)	100 (1)	100 (1)	100 (3)	100 (3)
5	5	100 (3)	100 (3)	100 (2)	100 (1)	100 (1)
6	6	100 (3)	100 (3)	100 (3)	100 (3)	100 (3)

Control

No.	Replication Number	1	2	3	4	5
1	1	-	-	-	-	-
2	2	-	-	-	-	-
3	3	-	-	-	-	-
4	4	-	-	-	-	-
5	5	+	-	-	-	-
6	6	-	-	-	-	-

+ Germinated - Not germinated

Second Observation

Treated

No.	Replication Number	1	2	3	4	5
1	1	100 (2)	100 (3)	100 (3)	100 (3)	100 (3)
2	2	100 (2)	100 (2)	100 (3)	100 (3)	100 (3)
3	3	100 (2)	100 (2)	100 (3)	100 (3)	100 (3)
4	4	100 (2)	100 (2)	100 (2)	100 (3)	100 (3)
5	5	100 (2)	100 (2)	100 (3)	100 (3)	100 (3)
6	6	100 (3)	100 (3)	100 (3)	100 (3)	100 (3)

Control

No.	Replication Number	1	2	3	4	5
1	1	-	-	-	-	-
2	2	-	-	-	-	-
3	3	-	-	-	-	-
4	4	-	-	-	-	-
5	5	+	-	-	-	-
6	6	-	-	-	-	-

\+ Germinated - Not germinated

TREATED AND WASHED

First Observation

Treated and Washed

No.	Replication Number	1	2	3	4	5
1	1	100 (1)	100 (1)	100 (1)	100 (1)	100 (2)
2	2	100 (1)	100 (1)	100 (3)	100 (3)	100 (3)
3	3	100 (1)	100 (1)	100 (2)	100 (3)	100 (3)
4	4	100 (3)	100 (3)	100 (1)	100 (1)	100 (1)
5	5	100 (2)	100 (3)	100 (3)	100 (3)	100 (3)
6	6	100 (3)	100 (3)	100 (3)	100 (2)	100 (1)

Control

No.	Replication Number	1	2	3	4	5
1	1	-	-	-	-	-
2	2	-	-	-	-	-
3	3	-	-	-	-	-
4	4	-	-	-	-	-
5	5	-	-	-	-	-
6	6	-	-	-	-	-

\+ Germinated - Not germinated

Second Observation

Treated and Washed

No.	Replication Number	1	2	3	4	5
1	1	100 (2)	100 (2)	100 (2)	100 (2)	100 (3)
2	2	100 (2)	100 (2)	100 (2)	100 (3)	100 (3)
3	3	100 (2)	100 (2)	100 (3)	100 (3)	100 (3)
4	4	100 (2)	100 (2)	100 (3)	100 (3)	100 (3)
5	5	100 (3)	100 (3)	100 (3)	100 (2)	100 (2)
6	6	100 (3)	100 (3)	100 (3)	100 (2)	100 (2)

Control

No.	Replication Number	1	2	3	4	5
1	1	-	-	-	-	-
2	2	-	-	-	-	-
3	3	-	-	-	-	-
4	4	-	-	-	-	-
5	5	-	-	-	-	-
6	6	-	-	-	-	-

+ Germinated - Not germinated

UNTREATED

First Observation

Untreated

No.	Replication Number	1	2	3	4	5
1	1	100 (3)	100 (3)	100 (2)	100 (1)	100 (1)
2	2	100 (1)	100 (1)	100 (2)	100 (3)	100 (3)
3	3	100 (1)	100 (1)	100 (3)	100 (3)	100 (3)
4	4	100 (3)	100 (3)	100 (1)	100 (1)	100 (1)
5	5	100 (1)	100 (1)	100 (1)	100 (2)	100 (3)
6	6	100 (2)	100 (1)	100 (1)	100 (1)	100 (1)

Control

No.	Replication Number	1	2	3	4	5
1	1	-	-	-	-	-
2	2	-	-	-	-	-
3	3	-	-	-	-	-
4	4	-	-	-	-	-
5	5	-	-	-	-	-
6	6	-	-	-	-	-

\+ Germinated - Not Germinated

Second Observation

Untreated

No.	Replication Number	1	2	3	4	5
1	1	100 (2)	100 (2)	100 (3)	100 (3)	100 (3)
2	2	100 (2)	100 (2)	100 (2)	100 (3)	100 (3)
3	3	100 (2)	100 (2)	100 (3)	100 (3)	100 (3)
4	4	100 (3)	100 (3)	100 (3)	100 (2)	100 (2)
5	5	100 (2)	100 (2)	100 (2)	100 (2)	100 (1)
6	6	100 (3)	100 (2)	100 (2)	100 (2)	100 (2)

Control

No.	Replication Number	1	2	3	4	5
1	1	-	-	-	-	-
2	2	-	-	-	-	-
3	3	-	-	-	-	-
4	4	-	-	-	-	-
5	5	-	-	-	-	-
6	6	-	-	-	-	-

+ Germinated - Not germinated

Average of percentages of visible area of sclerotia of *S. sclerotiorum* attacked by *Gliocladium* sp. in each treatment

No.	Treatment	First Observation	Second Observation
1	Treated	100.0	100.0
2	Treated and Washed	100.0	100.0
3	Untreated	100.0	100.0

Average of degrees of softness of sclerotia of *S. sclerotiorum* attacked by *Gliocladium* sp. in each treatment

No.	Treatment	First Observation	Second Observation
1	Treated	2.2	2.7
2	Treated and Washed	2.1	2.5
3	Untreated	1.8	2.4

Table No. 24 shows that:-

(1) All the sclerotia of both fungi were attacked by *Gliocladium* in all treatments (Figures 18 and 19).

(2) The percentage of visible area attacked by *Gliocladium* was almost 100% for both fungi in all treatments.

(3) All the sclerotia of both pathogens were soft. The degrees of softness were greater in the treated than in the untreated sclerotia.

(4) The degree of softness was greater in sclerotia of *S. sclerotiorum* than in those of *C. purpurea*.

(5) In the control plates of all treatments some sclerotia of *C. purpurea* were found germinating and producing the perfect stage. In control plates of 'treated' sclerotia of *S. sclerotiorum* only one sclerotium was observed germinating. Although not indicated in the

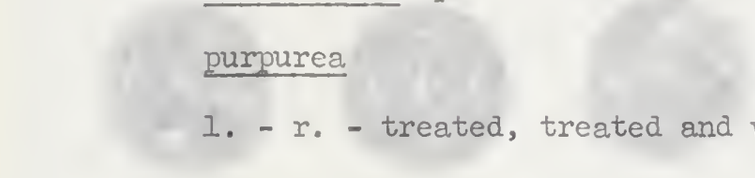

Fig. 18 Effect of artificial inoculation with *Gliocladium* sp. on sclerotia of *Claviceps purpurea*

l. - r. - treated, treated and washed and untreated

Fig. 18 Effect of artificial inoculation with *Ciliocladium* sp. on sclerotia of *Clavibacter tritici*. - T. - treated, treated and washed and untreated.

Fig. 19 Effect of artificial inoculation with Gliocladium sp. on sclerotia of Sclerotinia sclerotiorum

l. - r. treated, treated and washed and untreated

table it is noteworthy that no appreciable softening of the ungerminated sclerotia in the controls was observed.

The fungus which was attacking the sclerotia of C. purpurea and S. sclerotiorum in the inoculated plates was isolated and identified as a Gliocladium sp., according to the procedure described in Part III. Only two isolates were taken from each sclerotium.

It is concluded that Gliocladium sp. is parasitic on sclerotia of C. purpurea and inhibits its germination to the perfect stage. It also inhibits germination of sclerotia of S. sclerotiorum to the perfect stage.

The sclerotia of S. sclerotiorum in the control plates of all treatments grew and formed mycelium. On this mycelium new solid black bodies were formed. (Figure 20). These black bodies were surface sterilized by means of a dilute solution of "Perfex" (1 part in 10 parts of sterilized distilled water) and subsequently washed with sterilized distilled water. After this the "black bodies" were planted on sterilized solidified P.D.A. in Petri plates. Sclerotia of S. sclerotiorum obtained in the usual manner were given the same treatment as the black bodies and were planted as controls. It was found that the black bodies produced mycelium and in turn new sclerotia as did the sclerotia in the control plates. (Figure 21). This proved that the black bodies were sclerotia of S. sclerotiorum. The sclerotia which were formed on sterilized soil when the sclerotia of S. sclerotiorum were planted were called 'secondary' sclerotia. Their formation on sterilized soil has not been reported in the literature to the best of the writer's knowledge.

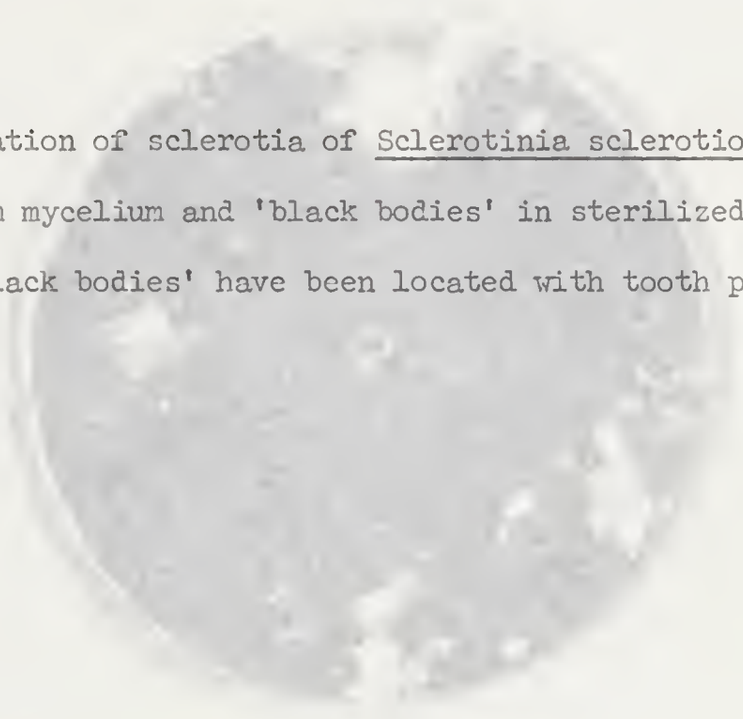

Fig. 20 Germination of sclerotia of Sclerotinia sclerotiorum to form mycelium and 'black bodies' in sterilized soil. The 'black bodies' have been located with tooth picks

Fig. 20 Germination of sclerotia of Sclerotinia sclerotiorum to form mycelium and 'black bodies' in sterilized soil. The 'black bodies' have been located with tooth picks

Fig. 21 Production of new sclerotia on potato sucrose agar from mycelium originating from 'black bodies' of Sclerotinia sclerotiorum formed in sterilized natural soil from normal sclerotia
The 'black bodies' are numbered 1, 2 and 3 in the Petri dish at the right, at the left a control culture originating from normal sclerotia is shown.

Fig. 21 Production of new sclerotia on potato sucrose agar from mycelium originating from 'black bodies' of *Sclerotinia sclerotiorum* formed in sterilized natural soil from normal sclerotia

The 'black bodies' are numbered 1, 2 and 3 in the Petri dish at the right, at the left a control culture originating from normal sclerotia is shown.

SUMMARY

(1) A comparative study of the behavior of the sclerotia of two phytopathogenic fungi, namely C. purpurea and S. sclerotiorum was made in soil and especially in natural soil.

(2) The sclerotia of C. purpurea used were collected from rye heads infected in the field while those of S. sclerotiorum were obtained from artificial agar cultures. In attempts to increase the yield of the latter various methods were investigated.

(3) Out of four media tested for sclerotia production by S. sclerotiorum, namely milk, cornmeal, potato dextrose and potato sucrose agars, milk agar was found the best and corn meal agar the poorest.

(4) The best temperature for the production of large numbers of sclerotia of S. sclerotiorum on milk agar was 20°C.

(5) In an attempt to get more sclerotia of S. sclerotiorum, glass beads, glass slides and glass tubes coated in the agar were used to increase growing surface in Petri dishes. Glass tubes proved most effective in increasing the yield and the size of the sclerotia.

(6) The behavior of sclerotia of C. purpurea was studied in natural and frozen soil. Treated (with $HgCl_2$), treated and washed and untreated sclerotia were planted. The sclerotia of all treatments were attacked by soil micro-organisms and the percentage of visible area of sclerotia attacked by soil micro-organisms was highest in the treated and lowest in the untreated group in both kinds of soil. No sclerotia germinated to perfect stage in any treatment and some sclerotia of the untreated group remained unattacked in natural soil. Some of the sclerotia which

remained unattacked in frozen soil, especially untreated ones germinated. The number of sclerotia attacked was less in frozen soil than in natural soil.

(7) The behavior of treated (with $HgCl_2$), treated and washed and untreated sclerotia of S. sclerotiorum was also studied in natural and frozen soil. The number of sclerotia attacked by soil micro-organisms was less in frozen soil than in natural soil and no sclerotia of the untreated group was attacked in both kinds of soil. The sclerotia showed the same trend of attack in all treatments as sclerotia of C. purpurea did. Certain unattacked sclerotia in all treatments were found germinating to the perfect stage.

(8) In comparative study of the behavior of sclerotia of both fungi under the same conditions it was found that the number of sclerotia and the percentage of visible area attacked by soil micro-organisms was less for S. sclerotiorum than for C. purpurea.

(9) The sclerotia of S. sclerotiorum 'degraded' in natural soil and 'black bodies' were formed on some of them in all treatments.

(10) The cut surfaces of sclerotia of both fungi were attacked by soil micro-organisms more readily than uncut surfaces in all treatments.

(11) Peeled sclerotia of C. purpurea were attacked more readily by the micro-organisms of natural soil in all treatments than unpeeled ones.

(12) Peeled sclerotia of S. sclerotiorum formed 'secondary' and 'pseudo' sclerotia around them in natural soil. This occurred in all treatments. The original peeled sclerotia degraded.

(13) One sclerotium out of 15 of C. purpurea from the treated and washed group degraded completely after 13 months following planting.

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(14) Two sclerotia out of 15 of C. purpurea from the treated group degraded completely after 7 months.

(15) The fungi, Gliocladium, Penicillium, Fusarium, Trichoderma and Aspergillus were found attacking the sclerotia of C. purpurea and S. sclerotiorum in natural and frozen soil.

(16) Uncontaminated sclerotia of C. purpurea were obtained by hot water treatment without impairing their viability. They were soaked for 1 - 6 hours before treating and 5 - 30 minutes was the time of treatment. Out of 5 temperatures tested i.e. 104°F., 112°F., 122°F., 131°F., and 140°F. it was found that 140°F. was the best temperature for treatment.

(17) In inoculation experiments using uncontaminated sclerotia Gliocladium sp. was shown to be parasitic on the sclerotia of C. purpurea and S. sclerotiorum and to prevent their germination to the perfect stage.

(18) The sclerotia of S. sclerotiorum germinated to form mycelium and to produce 'secondary sclerotia' when planted on sterilized soil.

DISCUSSION AND CONCLUSIONS

The two phytopathogenic fungi, namely Claviceps purpurea (Fries) Tulasne and Sclerotinia sclerotiorum (Lib) D By are responsible for destructive diseases of crop plants, which could be controlled in large measure, if the sclerotia produced by these pathogens could be destroyed or prevented from functioning. The present studies were undertaken with that end in view.

It has been found that when the sclerotia of C. purpurea were planted in natural soil especially following treatment with mercuric chloride they were attacked by soil micro-organisms and their germination was inhibited. They undergo degradation as a result of microbial attack. These findings confirm and extend those of Henry (8). The role of mercuric chloride in predisposing the sclerotia of C. purpurea to degradation may well have control value in the treatment of infested seed.

While sclerotia of S. sclerotiorum treated with mercuric chloride were also attacked by soil micro-organisms when planted in natural soil it was found that the number attacked was insignificant in comparison with the number of sclerotia of C. purpurea attacked. This together with the fact that some unattacked treated sclerotia of S. sclerotiorum were found germinating and that even degraded ones exhibited powers of regeneration by the formation of new sclerotia-like bodies in natural soil indicated that the role of mercuric chloride in predisposing the sclerotia of this fungus to the attack of soil micro-organisms is of minor importance.

From the fact that peeled sclerotia of C. purpurea in all treatments

were attacked more readily by soil micro-organisms than unpeeled ones in natural soil and that peeled sclerotia were degraded earlier than unpeeled ones it may be concluded that the black covering of the sclerotia of this fungus protects them against the attack of soil micro-organisms. It was noted in peeled sclerotia, too, that those treated with mercuric chloride were attacked and degraded by soil micro-organisms more readily than untreated ones. Thus the role of mercuric chloride is again emphasized.

The peeled sclerotia of S. sclerotiorum in all treatments degraded in natural soil but they germinated to 'secondary' and 'pseudo' sclerotia before they were completely degraded. Hence the peeled sclerotia of S. sclerotiorum did not behave like the peeled sclerotia of C. purpurea.

The probable reason why 'pseudo' sclerotia sometimes were formed by S. sclerotiorum in natural soil and why they did not become 'secondary' sclerotia was that sufficient nourishment could not be obtained where other micro-organisms were competing for it. Some sclerotia initials could obtain sufficient and some could not. Those able to obtain adequate nourishment probably became 'secondary' sclerotia while those only partially nourished remained 'pseudo' sclerotia. McNamara et al (1934) (10) have shown that the sclerotia of Phymatotrichum omnivorum (Shear) Dug. formed secondary sclerotia in the soil around the roots of cotton in natural soil. Such sclerotia probably obtained their nutrients from the roots of cotton plants and hence could compete with soil micro-organisms.

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